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SUPPLEMENTAL FEEDING OF PREGNANT EWES[1]

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In the fall of 1946 a co-operative experiment on sheep nutrition was organized by and under the sponsorship of the Project Group of the Sub-Committee on Nutrition of the National Sheep Committee. Institutions participating in this work were the Central Experimental Farm at Ottawa, Ontario; Dominion Experimental Station at Swift Current, Saskatchewan; Dominion Range Experiment Station, Manyberries, Alberta; and the Departments of Animal Husbandry at the University of Alberta, Edmonton, Alberta, and the University of Saskatchewan, Saskatoon, Saskatchewan. The project leader was P. E. Sylvestre.

The ewes were subject to the experimental treatment for the winter feeding period of 1946-1947 and the trial was repeated with some modifications and standardization of procedure during the season of 1947-1948. The results presented cover both of these periods.

REVIEW OF LITERATURE

One of the more important problems in sheep husbandry is that of overwintering the pregnant ewe on a ration which will combine economy with maximum productivity and which will ensure that the subsequent lamb crop is not adversely affected as measured by size, development and vigour. There is considerable evidence that the plane of nutrition of pregnant ewes does affect the size and vigour of their offspring and has an influence on the subsequent growth of their lambs. Hammond (2) found that in a flock in which the individual ewes were graded and classified as to condition at lambing, those ewes which carried the most flesh gave birth to heavier lambs than did those that were less well fleshed. Thompson and Fraser (12) reported heavier birth weights for both single and twin lambs when ewes were well fed throughout gestation and in addition found that equal results as measured by birth weights were obtained when the poor ration was replaced by a liberal diet one month before lambing. They also reported a relationship between lamb birth weights and vigour—the smaller, lighter lambs being weaker. Underwood and Shier (13) gave one-half pound of wheat per head per day for the last 4 to 6 weeks of pregnancy to a lot of ewes on grazing; a comparable check lot received no supplemental feeding. They found no difference in lamb birth weights under the two treatments but the small amount of supplement reduced the incidence of pregnancy toxaemia and lamb mortality. However, lambs that were heavier at birth grew faster; an increment of one pound in birth weight brought about a reduction of seven days in time required to reach a weight of between 65 and 70 pounds. Further work by these investigators (14), with higher levels of nutrition during the later stages of pregnancy, showed highly significant differences in birth weights in favour of the heavier rate of feeding. Subsequent lamb growth was better for the fed lot but this increased growth rate could be wholly accounted for by the increase in birth weight. They concluded that under their conditions very high levels of prenatal feeding are unlikely to be economic since the increase in growth rate from such treatment is not, on the present evidence, very great and losses of lambs and ewes can be prevented equally as well by a small supplement of grain. Wallace (15) (16) reported that the prenatal plane of nutrition markedly affects birth weights. He also reported a high correlation (0.7276) between birth weight and live weight increase to 28 days and that lamb vigour and viability were influenced profoundly by the plane of nutrition of their dams.

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Since in many areas of this country legume hay cannot be grown, or if it is grown it is usually reserved for the dairy herd, it was proposed to determine the best method of utilizing roughage for pregnant ewes. Trials conducted at the Central Experimental Farm, Ottawa (11) have shown that timothy unsupplemented is not a suitable feed for pregnant ewes in that there is a heavy loss in body weight of the ewes, a high percentage of still-born lambs, a low percentage of good lambs, and a high subsequent lamb mortality.

Various workers have shown that the vitamin A content of timothy hay is lower than that of legume hay (6), (17) and it has been demonstrated that ample vitamin A must be provided for normal reproduction and normal health of the new born (3), (4), (5), (10). It was therefore decided to have three lots under test—one on legume hay, one on non-legume hay and one on non-legume hay changed to legume hay plus grain at mid-pregnancy. In addition, half of each of these lots would receive a vitamin A supplement.

PROCEDURE

An experimental outline was drawn up and was used by each Station. While in certain cases there was some variation between the co-operators these cases will be noted throughout the report as they arise. In general the procedure was as follows: A flock of ewes of the same or similar breeding was selected; these ewes were all bred to one ram or, if two rams were used, they were of similar breeding. During the breeding season all ewes received the same treatment with regard to feeding and management. Ochre was used on the rams and as soon as the ewes were judged to be in lamb they were allotted to three lots on the basis of breed, age, weight, condition and ram to which they had been bred. The treatment of the lots was then as follows:

Lot I—Legume hay plus mineral mixture.

Lot II—Non-legume hay plus mineral mixture.

Lot III—Non-legume hay plus mineral mixture from time of allotment until 100 days after the time the rams were put with the ewes; then legume hay plus mineral mixture plus a supplementary allowance of grain in the amount necessary to cause a steady gain in the live weight and condition of the ewes.

In addition, half of each lot received a weekly allowance of 50,000 I.U. of vitamin A. This was administered orally in capsule form and consisted of fortified high potency feeding oil. Roughage and minerals were fed ad libitum. All feeds were fed from racks or troughs.

The mineral supplement consisted of the following mixture:

50	pounds feeding bonemeal
50	pounds salt
0.8	ounces cobalt sulphate
0.5	ounces manganese sulphate
0.3	ounces copper sulphate
4.0	ounces ferrous sulphate
2.4	ounces stabilized potassium iodide.

Animals of each lot were penned together and bedded with straw. Access was given to a yard for exercise. Water was provided at least twice daily.

Lamb Birth Grades. All lambs were weighed and graded within 24 hours of birth. At the same time the sex and birth state (i.e. twin, single) of the lamb was recorded. The following grade standards were used:

(a) *Good*—Lambs of good weight, strong and which appear to have excellent chances of survival. Lambs in this category do not require any assistance at nursing.

(b) *Fair*—Lambs of good weight and fairly strong. These lambs should appear to have a good chance of survival. They may exhibit a little softness or sleepiness as opposed to brightness or life. They may require assistance at nursing for the first day.

(c) *Weak*—Soft, weak or small lambs. These lambs require assistance at nursing for a considerable period or may need extra heat and bottle feeding. Their chances of survival would appear to be negligible without the extra assistance. Whenever possible the cause of this grading was recorded.

(d) *Dead*—All lambs born dead or which die within a few hours of birth from causes other than accident. All defects and any evidences of abnormality or disease in the lambs or ewes were recorded.

Numbers, Breeds Used and Rations Fed

For convenience in preparing the tabular data all co-operators have been allotted a code letter and each institution will be identified in all subsequent tables by its code letter.

Institution	Code letter
Dominion Experimental Station, Swift Current, Sask.	A
Dominion Range Experiment Station, Manyberries, Alta.	B
University of Saskatchewan, Saskatoon, Sask.	C
University of Alberta, Edmonton, Alberta	D
Central Experimental Farm, Ottawa, Ontario	E

It will be noted that no data are presented for Station A for the season of 1947-1948 as this Station was unable to conduct the trial during that period.

In all, a total of 396 ewes were involved in the trial, 204 head in 1946-1947 and 192 head in 1947-1948. The distribution of these animals is shown in Table 1.

TABLE 1.—NUMBER OF EWES PER GROUP AT EACH STATION

Station	Lot I		Lot II		Lot III	
	Vit. A	No vit.	Vit. A	No vit.	Vit. A	No vit.
1946-1947						
A	5	5	5	5	5	5
B	5	5	5	5	5	5
C	7	7	7	7	7	7
D	8	8	8	8	8	8
E	9	9	9	9	9	9
Total 1946-1947	34	34	34	34	34	34
1947-1948						
B	8	8	8	8	8	8
C	8	8	8	8	8	8
D	7	7	7	7	7	7
E	9	9	9	9	9	9
Total 1947-1948	32	32	32	32	32	32
Total of both years	66	66	66	66	66	66

Over the two years there were available for comparison of the vitamin A treatment versus no vitamin A within each lot, 66 head; for the comparison of the various rations, 112 head; and for the comparison of the value of vitamin A over the three lots, 198 head.

The breeds of sheep used at each Station were as follows:

1946-1947

A—Rambouillet ewes bred to Romeldale ram
B—Romnelet ewes bred to Romnelet ram
C—Rambouillet ewes bred to Rambouillet ram
D—Suffolk, Hampshire and Romnelet ewes in the proportion of 6, 4, 6 bred to rams of their respective breeds
E—Grade Shropshire ewes bred to Shropshire ram.

1947-1948

B—Romnelet ewes bred to Romnelet ram
C—Rambouillet ewes bred to Rambouillet ram
D—Suffolk ewes bred to Suffolk ram
E—Grade Shropshire ewes bred to Shropshire ram.

The composition of the rations fed along with the amounts fed per head per day at the various Stations is shown in Tables 2A and 2B. At the foot of each table are notes indicating in a general way the type and quality of the various feeds.

While not all co-operators were able to obtain a chemical analysis of the feeds used during the first year of this trial, in the second year more complete data were obtained. Table 3 gives these figures and it will be noted that while the roughages fed at the various Stations differed quite widely in type, the difference as shown by these figures between the legume and non-legume hays is, with the exception of one Station, fairly constant.

As might be expected from the widely divergent geographical location of the co-operators, the dates of starting the trial, dates of lambing, etc., showed quite a wide range. As there was a change in procedure between the two years, these dates are reported in two tables, 4A and 4B. In the first year of the experiment several co-operators discontinued the feeding

TABLE 2A. COMPOSITION OF RATIONS AND AMOUNT FED PER HEAD PER DAY, 1946-1947

Station	Lot I	Lot II	Lot III
A. 1st period	Alfalfa hay, 4.1 lb.	Oat sheaves, 4.3 lb.	Oat sheaves, 3.8 lb.
2nd period	Alfalfa hay, 4.1 lb.	Oat sheaves, 4.3 lb.	Alfalfa hay, 4.7 lb. Oats, 0.75 lb.
B. 1st period	Alfalfa hay, 5-8 lb.	Timothy ¼, Crested wheat grass ¾, 5-8 lb.	Timothy ¼, Crested wheat grass ¾, 5-8 lb.
2nd period	Alfalfa hay, 5-8 lb.	Timothy ¼, Crested wheat grass ¾, 5-8 lb.	Alfalfa hay, 5 lb. Oats ¾, Oilcake ¼, 1.0-1.5 lb.
C. 1st period	Alfalfa hay, 4 lb.	Oat sheaves, 4 lb.	Oat sheaves, 4 lb.
2nd period	Alfalfa hay, 4 lb.	Oat sheaves, 4 lb.	Alfalfa hay, 3.25 lb. Oats, 0.75 lb.
D. 1st period	Alfalfa hay, 6.25 lb.	Oat and brome hay, 6.25 lb.	Oat and brome hay, 6.25 lb.
2nd period	Alfalfa hay, 6.25 lb.	Oat and brome hay, 6.25 lb.	Alfalfa hay, 6.25 lb. Oats, 0.5 lb.
E. 1st period	Clover hay, 2.7 lb.	Timothy hay, 2.7 lb.	Timothy hay, 2.7 lb.
2nd period	Clover hay, 2.7 lb.	Timothy hay, 2.7 lb.	Clover hay, 2.7 lb. Oats ⅜, bran ⅜, Malt sprouts ⅛—0.5 to 1.1 lb.

Notes regarding quality:

A. Alfalfa hay—good quality
Oat sheaves—good quality, contained considerable grain.
Oat sheaves were put through a hammer mill

B. Alfalfa hay—good quality
Non-legume hay—good quality

C. Alfalfa hay—good quality
Oat sheaves—fair quality

D. Alfalfa hay—first cut was fed for first 50 days; then second cut containing about 25 per cent timothy was fed
Brome hay—good quality

E. Clover hay—fair quality. From April 8 on, second cut alfalfa hay of good quality was fed in place of clover hay
Timothy hay—good quality, bright and green.

TABLE 2B.—COMPOSITION OF RATIONS AND AMOUNT FED PER HEAD PER DAY, 1947-1948

Station	Lot I	Lot II	Lot III
B. 1st period	Alfalfa hay, 4 lb.	Crested wheat grass hay, 3.9 lb.	Crested wheat grass hay, 3.9 lb.
2nd period	Alfalfa hay, 4 lb.	Crested wheat grass hay, 4.0 lb.	Alfalfa hay, 4.0 lb. Oats, 0.93 lb.
C. 1st period	Alfalfa hay, 4 lb.	Oat sheaves and Meadow hay, 5.0 lb.	Oat sheaves and Meadow hay, 5.0 lb.
2nd period	Alfalfa hay, 4 lb.	Oat sheaves and Meadow hay, 5.0 lb.	Alfalfa hay, 4.0 lb. Wheat screenings, 0.5 lb.
D. 1st period	Alfalfa and Timothy hay, 5.21 lb.	Brome hay, 4.86 lb.	Brome hay, 4.90 lb.
2nd period	Alfalfa and Timothy hay, 5.29 lb.	Brome hay, 5.03 lb.	Alfalfa and Timothy hay, 4.64 lb., Oats, 0.5 lb.
E. 1st period	Clover and Alfalfa hay, 2.98 lb.	Timothy, 3.01 lb.	Timothy, 3.00 lb.
2nd period	Clover and Alfalfa hay, 3.11 lb.	Timothy, 3.42 lb.	Clover and Alfalfa hay, 3.09 lb. Oats, 0.92 lb.

Notes regarding feed quality:

B. Alfalfa hay—first cut, sun-cured and baled
Crested wheat grass hay—sun-cured and loosely stacked
90 per cent crested wheat grass, 10 per cent wild barley, spear grass and sweet clover

C. Alfalfa hay—fair quality
Oat sheaves—good quality
Meadow hay—fair quality

D. Alfalfa and timothy hay—fair quality, 50 per cent alfalfa
Brome hay—good quality
Oats—feed oats weighing about 38 pounds per bushel

E. Clover and alfalfa hay—fair quality mixed clover and alfalfa with about 25 per cent timothy, field baled in round bales
Timothy hay—fair quality with from 10 to 20 per cent clover
Oats—good quality feed oats.

of the experimental rations as soon as the first ewe lambed, while in 1947-48 all ewes were kept on the experimental ration until they had lambed with the exception of those of co-operator "C", these sheep being removed from the trial at the end of March.* Lambing in this group started on April 12. In all lots the date of change-over in the ration of Lot III was also the date on which the intermediate weighing of the ewes took place. Thus Stations showing the longest period from start to date of change-over could also be expected to show the greatest differences in ewe body weight at this time. In Tables 4A and 4B "Init." refers to the date on which the ewes were started on the experimental rations and "Inter." is that on which the ration of Lot III was changed to legume hay plus grain. In Table 4A the last columns refer to the actual number of days to first lambing while the same columns in Table 4B contain the average number of days to the actual lambing date of each ewe.

TABLE 3.—CHEMICAL COMPOSITION OF FEEDS IN PER CENT

Station	Feed	Protein	Ether extract	Crude fibre	Ash	N.F.E.
1946-1947						
A.	Alfalfa hay	16.1	—	32.0	—	—
	Oat sheaves	9.7	—	32.4	—	—
E.	Clover hay	13.3	0.9	35.5	6.4	43.9
	Timothy hay	8.5	0.9	43.7	5.7	41.1
	Grain mixture	16.7	4.0	13.2	4.9	61.2
1947-1948						
B.	Alfalfa hay	14.1	0.6	44.6	6.2	34.6
	Crested wheat hay	9.9	1.3	31.4	8.3	49.2
	Oats	16.1	4.4	13.0	3.6	62.8
C.	Alfalfa	14.6	2.1	27.1	8.6	—
	Oat sheaves	8.4	3.7	22.4	4.6	—
	Meadow hay	5.7	2.1	31.2	5.6	—
	Wheat screenings	15.5	2.8	3.6	2.3	—
D.	Alfalfa and Timothy hay	13.0	2.1	36.4	6.5	36.6
	Brome hay	12.2	2.0	28.4	5.0	48.0
	Oats	12.7	6.2	11.5	3.3	61.3
E.	Clover and Alfalfa hay	10.7	0.9	42.3	5.7	40.4
	Timothy hay	7.2	1.3	39.9	5.3	46.2
	Oats	14.6	4.9	13.0	3.3	64.1

TABLE 4A.—LENGTH OF PERIODS, 1946-1947

Station	No. days Init. to Inter.	No. days Inter. to Lamb.	No. days Init. to Lamb.
A	61	40	101
B	34	77	111
C	33	52	85
D	77	68	145
E	53	40	93
Average	52	55	107

TABLE 4B.—LENGTH OF PERIODS, 1947-1948

Station	No. days Init. to Inter.	Av. no. days Inter. to Lamb.	Av. no. days Init. to Lamb.
B	56	56	112
*C	100	37	137
D	63	57	120
E	54	68	122
Average	68	54	123

* "Init." was the start of the breeding season and "Lamb." was the date when the ewes were removed from e experimental ration.

EXPERIMENTAL RESULTS

Although the original outline of the trial did not call for post-natal mortality records, as there were indications that there might be differences in mortality rates, particularly between vitamin A and no vitamin A treatment, all Stations were asked to submit their mortality figures. Data were obtained from all but one Station and are summarized in Table 5.

TABLE 5.—LAMB MORTALITIES 1947 AND 1948

	Lot I		Lot II		Lot III		All lots	
	Vit. A	No A	Vit. A	No A	Vit. A	No A	Vit. A	No A
Live births	85	81	82	74	82	83	249	238
Mortality:								
To 28 days	13	18	15	15	14	17	42	50
To weaning	18	26	21	25	17	20	56	71
Per cent mortality:								
To 28 days	15	22	18	20	17	20	17	21
To weaning	21	32	26	34	21	24	22	30
Per cent mortality of lot:								
To 28 days	19		19		19		19	
To weaning	26		30		22		26	

While the differences in mortality rates seem consistent and in line with what might be expected, yet in no case do they approach significance. This is doubtless due to the tremendous range in percentages both between and within Stations and lots (0 to 73 per cent).

Table 6 presents the results for 1947 and 1948 as well as a summary of the two years.

DISCUSSION

Due to the nature of the data and the widely different feeds and environmental conditions to which the lots were subjected at the different locations, a statistical analysis of the data has not been carried out. The following points, however, were common to all Stations and justify certain conclusions:

(a) At each Station there were three lots; one received a legume hay, one received a non-legume hay and the third received non-legume hay until approximately mid-pregnancy and from then on was given legume hay plus a grain supplement.

(b) At each Station half of each lot received a high potency vitamin A supplement.

(c) All sheep were allowed free access to a mineral supplement.

The following observations are made:

(a) Inspection of Table 6 shows that legume hay plus minerals gave more satisfactory results when fed to pregnant ewes than did non-legume hay. Ewes fed legume hay maintained their weight more satisfactorily and gave birth to heavier, more vigorous lambs. Although there is a

TABLE 6.—SUPPLEMENTARY FEEDING OF PREGNANT EWES. ALL STATIONS. SUMMARY 1947 AND 1948

	Lot I Legume			Lot II Non-Legume			Lot III Changeover			All	
	Vit. A	No Vit. A	All	Vit. A	No Vit. A	All	Vit. A	No Vit. A	All	Vit. A	No Vit. A
1947											
No. ewes	34	34	68	34	34	68	34	34	68	102	102
Av. gain Init.—Lamb.	−6.7	−4.6	−6.5	−11.2	−11.5	−11.4	−7.9	−6.6	−7.3	−8.5	−7.6
No. ewes died	0	1	1	3	1	4	0	0	0	3	2
No. ewes lambed	31	27	58	28	26	54	28	30	58	87	83
No. live births	54	44	98	39	36	75	40	44	84	133	124
No. dead births	3	1	4	3	3	6	1	0	1	7	4
Per cent "Good" lambs	74	67	71	64	56	60	80	93	87	73	73
Av. birth weight	8.1	8.1	8.1	8.8	8.1	8.5	8.7	9.1	8.9	8.6	8.4
Lambing per cent	174	163	169	139	138	139	143	147	145	153	149
1948											
No. ewes	31	32	63	32	32	64	32	32	64	95	96
Av. gain Init.—Lamb.	−1.8	−4.5	−3.2	−18.3	−13.0	−15.6	−3.6	−3.5	−3.5	−8.1	−7.0
No. ewes died	1	1	2	0	1	1	0	0	0	1	2
No. ewes lambed	28	30	58	31	31	62	30	31	61	89	92
No. live births	41	43	84	51	43	94	47	45	92	139	131
No. dead births	1	2	3	2	3	5	2	1	3	5	6
Per cent "Good" lambs	74	69	71	58	56	58	77	65	72	69	63
Av. birth weight	8.2	8.3	8.3	7.3	7.5	7.4	8.5	8.5	8.5	8.0	8.1
Lambing per cent	146	143	145	164	139	152	157	145	151	156	142
1947 and 1948											
No. ewes	65	66	131	66	66	132	66	66	132	197	198
Av. gain Init.—Lamb.	−4.4	−4.6	−4.5	−14.6	−12.2	−13.4	−5.8	−5.1	−5.5	−8.3	−7.3
No. ewes died	1	2	3	3	2	5	0	0	0	4	4
No. ewes lambed	59	57	116	59	57	116	58	61	119	176	175
No. live births	95	87	182	90	79	169	87	89	176	272	255
No. dead births	4	3	7	5	6	11	3	1	4	12	10
Per cent "Good" lambs	74	68	71	61	56	59	79	79	79	71	68
Av. birth weight	8.1	8.2	8.2	8.0	7.8	7.9	8.6	8.8	8.7	8.2	8.3
Lambing per cent	161	153	157	153	139	146	150	146	148	155	146

difference in lambing percentage in favour of the lot fed legume hay, assuming a coefficient of variability in lambing percentage of 35 per cent, this difference is less than is required for significance. This is to be expected since the ewes were not—with the exceptions previously noted—put on the experimental rations until after breeding and normally eggs shed by the ovulating ewe develop successfully unless they are not, as sometimes happens, fertilized or implanted (8). Thus the treatments should not have affected the lambing percentages.

(b) Under the conditions of the experiment the addition of a vitamin A supplement to any of the rations did not improve the ration as measured by maintenance of ewe body weight, or birth weight and vigour of the lambs. While once again there were differences in mortality rates and lambing percentages between the lots, these can be explained on the basis of normal variability. This is in agreement with the findings of Hart and Miller (4) who reported that reproduction was not impaired in sheep unless the ewes were vitamin A-depleted before the start of a winter feeding period trial. While carotene analyses were obtained from only one Station (E), it is of interest to note that these show that even the ewes on the non-legume roughage were receiving two to three times their normal requirements (1), (7), (9) of this vitamin.

SUMMARY

During the winter of 1946-47 five institutions co-operated in conducting an experiment with pregnant ewes under a common project outline. At each institution the following lots were fed: *(a)* legume hay throughout pregnancy; *(b)* non-legume hay throughout pregnancy; *(c)* non-legume hay until mid-pregnancy, then legume hay plus grain. Half of each lot received a high potency vitamin A supplement. The trial was repeated the following winter. Under the conditions of the experiment:

(a) Legume hay gave more satisfactory results than did non-legume hay as measured by maintenance of ewe body weight throughout pregnancy, lamb birth weight and lamb vigour at birth.

(b) The change-over ration gave results comparable to those obtained when legume hay alone was fed.

(c) The addition of a vitamin A supplement to these rations did not bring about any significant improvement.

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CORRELATION STUDIES OF YIELD AND OTHER CHARACTERS IN RYE POLYCROSSES[1]

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Although important in the economy of the United States, rye has not generally been subjected to an intensive improvement program. The desirability of such a program is indicated by the uniformity of the average yields during a period when the average yields of other crops, notably corn and wheat, have been rising steadily. Also worthy of note is the rarity of introduction of new improved rye varieties.

Several reasons account for the lack of emphasis on rye breeding. The mode of pollination presents some special problems. Rye is cross-pollinated so that breeding by selfing, selection and crossing should be effective. However, self-sterility complicates the process of inbreeding and the rather high proportion of unfruitful florets after natural cross-pollination offers another difficulty.

Present world demands for food and feed, however, are so insistent that investigation of the possibilities presented by new breeding methods applicable to rye is of considerable interest. One such breeding method, being studied at the University of Minnesota, is the use of the polycross for testing the performance of rye inbreds and for the subsequent combining of selected progenies.

The methods used in these studies consisted of the selection of plants that had a relatively high seed-setting when self-pollinated. Selection was made also for desirable seed and plant characters. The selected lines were combined in two polycross nurseries, one containing lines relatively homozygous for seeds with colourless aleurone and the other, lines homozygous for green aleurone. The performance in polycross yield trials was studied in relation to the length of time that the lines had been self-pollinated and to the extent of self-fertility of the inbreds. A major purpose of this investigation was to make an intensive study of the characters of the polycrosses in comparison with standard varieties. On the basis of these results various combinations of inbreds were selected for the production of synthetic varieties.

REVIEW OF LITERATURE

Earlier investigations with rye are of interest in relation to this study. Previous reports by Brewbaker (1) and Peterson (4) in Minnesota have shown that self-fruitful plants can be obtained sufficiently frequently to make selection in self-pollinated lines possible for seed and plant characters and to produce seed in sufficient quantity for polycross studies. Peterson's results indicate that self-fertility vs. self-sterility is not a simply inherited character in rye; frequently, in the early generations of selfing, both self-fertile and self-sterile plants are obtained in the progeny of a self-pollinated plant that itself was high in seed setting under conditions of self-pollination. Leith and Shands (3) also have studied the effects of long continued selfing. They, like Brewbaker, suggested compositing selfed lines to produce synthetic varieties. A variety recommended by the Minnesota station, named Emerald, was produced in this manner. Krasniuk (2) reports a rye inbred line that after six generations of selfing yielded 46 per cent more than the local rye variety.

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The polycross method of determining combining ability in alfalfa was originally described by Tysdal, Kiesselbach and Westover (6). The selected clones were grown together in a single nursery and so randomized that outcrosses of these would be effected by a reasonably comparable sample of pollen. Seed was bulked from all replicates of the same clonal line and used in a performance trial. Better combiners, as indicated by their performance, were selected to produce a synthetic. Other advantages of the method were stated to be (*a*) ample seed produced for testing; (*b*) self-sterile lines could be tested effectively; (*c*) inbreeding was not required.

Tysdal and Crandall (5) later compared the polycross method with top cross and average single cross performance of the same eight clonal lines as a means of selecting high combining clones in alfalfa. For the four characters studied, including forage yield, cold resistance, bacterial wilt resistance, and leaf hopper resistance, the eight clones ranked practically the same by each method. The synthetic variety resulting from recombining clones selected on the basis of polycross performance was significantly higher in yield than the standard varieties or the synthetic composed of low combining clones selected by the same method.

MATERIALS AND METHODS

The selfed lines selected to be grown in the polycross crossing plots were those that had medium-to-high seed production under conditions of self-pollination, when several heads of a plant were isolated in vegetable parchment bags. Only the more vigorous selfed plants and lines were selected. Selection was only on a plant basis for the lines that originated from plants of Dakold and Imperial that had been selfed only for the first time. Selfed plants were selected for the polycross crossing plots that excelled in seed size, plumpness and quality of seed, and that appeared to be breeding relatively true for either colourless or green seed colour.

Each polycross crossing plot was made up of individual plant hills six inches apart, in rows one foot apart, arranged in a randomized block of thirty replications. The thirty hills of each inbred were thus associated in a random manner with the other inbreds in the nursery. Every plant should have been pollinated with a random selection of pollen from other lines in the nursery except for differences resulting from variations in time of shedding pollen, differential viability, cross sterility, and other similar causes. The individual plants of each line were harvested together, threshed and the seed bulked for seeding in the performance trials. Originally, there were 64 colourless aleurone lines in Polycross Nursery I and 23 green aleurone lines in Polycross Nursery II. The yield trials and study of characters of polycrosses were made from 30 colourless lines and 13 green aleurone lines. These are the lines that appeared relatively homozygous

TABLE 1.—ORIGIN, YEARS SELFED, CLASSIFICATION FOR SEED COLOUR AND FOR SELF-FERTILITY OF SELFED LINES OF RYE USED IN POLYCROSS YIELD TRIALS

Polycross line No.	Seed colour	Origin	Years selfed	Fertility group
2	Colourless	(High × Low) × Minn. 111	5	1
3	Colourless	High fertility	19	1
4	Colourless	Sagnitz	16	2
6	Colourless	Dakold	8	1
8	Colourless	Dakold	5	1
10	Colourless	Dakold	4	2
12	Colourless	Dakold	3	2
17	Colourless	Dakold	3	3
21	Colourless	Dakold	1	4
22	Colourless	Dakold	1	4
24	Colourless	Dakold	1	3
25	Colourless	Dakold	1	2
29	Colourless	Dakold	1	5
30	Colourless	Dakold	1	2
31	Colourless	Dakold	1	4
34	Colourless	Dakold	1	4
35	Colourless	Dakold	1	4
37	Colourless	Dakold	1	4
41	Colourless	Dakold	1	4
48	Colourless	Imperial	1	5
50	Colourless	Imperial	1	3
51	Colourless	Imperial	1	3
52	Colourless	Imperial	1	3
53	Colourless	Imperial	1	4
55	Colourless	Imperial	1	3
56	Colourless	Imperial	1	3
58	Colourless	Imperial	1	4
59	Colourless	Imperial	1	4
61	Colourless	Imperial	1	3
64	Colourless	Imperial	1	4
13	Green	Dakold	3	3
65	Green	Dakold	5	2
66	Green	Dakold	8	2
67	Green	Dakold	5	2
71	Green	Dakold	3	5
72	Green	Dakold	2	5
73	Green	Dakold	2	2
74	Green	Dakold	1	5
75	Green	Dakold	1	5
76	Green	Dakold	1	3
77	Green	Dakold	1	5
78	Green	Dakold	1	3
81	Green	Dakold	1	3

for seed colour on rechecking, that appeared most desirable as inbreds, that produced sufficient seed for the yield trials and for which sufficient selfed seed was available to continue the line.

The inbred lines grown in the polycross yield trials are listed in Table 1. The origin, generations selfed when first grown in the polycross nurseries and classification of lines for self-fertility are given.

Classification for self-fertility was based on 1947 data for lines listed as selfed only once, while lines selfed for a longer period were studied in both the 1946 and 1947 crop seasons. They were placed in five self-fertility groups as follows: (1) all plants highly self-fertile; (2) nearly all plants highly self-fertile with an occasional self-unfruitful plant; (3) approximately one-half of plants highly self-fertile; (4) approximately one-fourth plants highly self-fertile; (5) all plants medium-to-low in self-fertility. In group 5 it was necessary to combine seed from several plants.

The performance of the polycrosses was tested in two groups arranged to include nearly equal numbers of lines. Group I was made up of twenty-one lines of which nine were obtained from the colourless selections and twelve from the green selections in the polycross plots. Group II was made up of twenty-two lines from the colourless selections. The individual lines, selected to be grown in the polycross yield trials, were those that had no extremely undesirable plant characters in the polycross crossing plots. They also produced sufficient seed for the yield trials and produced grain of comparatively good quality, size and plumpness. The three standard varieties recommended for Minnesota—Dakold, Acc. No. 93, Imperial, Acc. No. 115, and Emerald, Acc. No. 93—were included in each group.

Each polycross line and variety was planted, when sufficient seed was available, in single rod-row plots replicated six times. In Group I, five lines were replicated only three times and two others were replicated four times because of insufficient seed. Rate of seeding was five hundred seeds per eighteen-foot row, and the rows were spaced one foot apart. The two groups of polycrosses were planted in adjacent areas on September 13, 1946. Replications for Polycross Group I were sown in rows 1 to 128, inclusive and those for Polycross Group II in rows 129 to 278, inclusive.

Planting conditions were good and first emergence was noted on September 17. The stand of most of the polycross progenies was equal to that of the standard varieties. Fall growth vigour ratings were made on October 21, 1946, on the basis of the appearance of the rows. Classes of one to five were used with one representing poor vigour and five very good vigour. At this time, most plants appeared in good condition to survive the winter.

Winterkilling was negligible, and in the spring most lines made rapid early growth. Stooling vigour was rated in the first week of May in classes of one to five. A rating of five represented very good stooling vigour, while a rating of one indicated poor stooling vigour.

Plants of many of the polycross progenies began to head out during the latter part of May. A row was recorded as headed when approximately fifty per cent of the plants in the row had produced heads. The majority of the polycrosses were in head by the end of the first week of June. Height at maturity was measured as the average for the row and the uniformity of height was also estimated. Since all rows were classified as eighty per cent or better in uniformity, only the average height for each row was recorded.

At maturity, a one-foot border of plants was cut off each end of every row and discarded. Each plot, consisting of a 16-foot row, was harvested and threshed separately. Yields in grams of individual rows were converted to yields in bushels per acre by the use of a factor.

During the harvesting operation, a sample of twenty-five normal heads was collected at random from each row and placed in an envelope for later use in the head and kernel studies. The total number of lateral florets, exclusive of those in the lowermost and terminal spikelets which rarely produce seeds, was counted for each of ten heads from each row. The seeds from the ten-head sample were then rubbed out, counted, and the total number of seeds produced was expressed as a percentage of the total number of florets for per cent fruitfulness. The weight in grams of this known number of kernels was determined and the weight per hundred kernels was calculated.

Studies were made on pollen sterility of the polycrosses during two seasons. The per cent of quartets with micronuclei during microsporogenesis was also studied. For each pollen sample an entire head was taken, labelled and stored in 70 per cent alcohol for subsequent pollen sterility determinations. Sterility was estimated in the following manner. A whole anther was removed from a floret near the region where the anthers were extruded and its entire contents squeezed into a strong potassium iodine solution. Pollen grains of normal size and more than one-half filled with starch were considered normal and all others as abortive. The per cent sterility was estimated with reference to specially prepared slides showing various degrees of sterility verified by counting large numbers of pollen grains. Pollen sterility was estimated on at least two separate occasions for each sample, the average of these estimations being used in the analysis.

Sporocyte material was collected and placed in vials containing freshly prepared killer solution made up of three parts of 95 per cent alcohol and one part of glacial acetic acid. The samples were immediately stored in a refrigerator at about 36° F. After thirty-six hours, the killer solution was replaced by 70 per cent alcohol and the samples returned to the refrigerator.

Microsporogenesis was studied by means of the iron aceto-carmine smear technique. Smears were made of complete anthers in the various stages of meiosis in an attempt to locate irregularities of division which might lead to pollen or ovule sterility and, in turn, influence yield.

Particular emphasis was placed on the examination of spore quartets for micronuclei. The quartet stage was located and smears prepared from two anthers, wherever possible from different florets, of each plant. In each preparation, approximately two hundred quartets were classified according to the number of micronuclei present. The per cent of quartets with micronuclei was then calculated.

Analyses of variance to determine the significance of varietal differences were calculated for Group I and Group II separately for eight characters, namely, fall growth vigour; stooling vigour; date of heading; height of plant; weight per bushel; yield in bushels per acre; per cent fruitfulness, and weight of one hundred kernels. Per cent data were converted to degrees of $\sin^2 \theta$ before analysis. The significance of varietal differences in pollen sterility was determined by analysis of variance using data for the two years as replicates.

Simple product moment correlation coefficients were calculated to determine the degree of association between yield and the other agronomic characters. On the basis of these correlation coefficients, eight characters, namely, yield; fall growth vigour; stooling vigour; date of heading; weight per bushel; per cent pollen sterility; per cent fruitfulness, and weight per hundred kernels, were selected for further correlation studies.

Correlation coefficients were calculated for all interrelationships of these eight characters. Utilizing these simple product moment correlation coefficients, the partial correlation coefficients for all combinations of the eight characters were calculated. The multiple correlation for the dependence of yield on the other seven characters was then determined.

After learning the relative value of various seed and plant characters in relation to yielding ability, the data obtained were used in selecting inbred lines for the production of several synthetic varieties.

In reporting the statistical results of this study, a single asterick (*) is used to indicate significance at the five per cent level and a double asterisk (**) to indicate significance at the one per cent level.

EXPERIMENTAL RESULTS

Characters of Polycrosses in Relation to Three Standard Varieties

Agronomic Characters

There were twenty-one polycrosses and three varieties grown in the yield trials for Group I and twenty-two polycrosses and the three varieties in yield Group II. Separate analyses of variance were made for Groups 1 and 2, respectively. Differences between entries were highly significant in both groups for the following characters: fall growth vigour; stooling vigour; date of heading; height of plant; weight per bushel; per cent fruitfulness; and yield in bushels per acre. Differences between entries for weight of 100 kernels was highly significant in yield Group II and significant at the 5 per cent level in Group I.

As the three recommended varieties, Dakold, Emerald and Imperial, were grown in each of the two yield trials, they were used as a basis for the study of characters of the polycrosses grown in the same yield group. In Table 2 the frequency distribution of twenty-one polycrosses in Group I and twenty-two polycrosses in Group II is shown in relation to the mean of the three standard varieties grown with each group. Class centres of the distribution are separated by multiples of the least significant difference at the five per cent level, except for class 0, and are arranged in plus and minus classes on each side of the zero class. The zero class contains all means that did not differ from the average of the three varieties by as much as plus one or minus one times the least significant difference. Class + 1.5 contains all means whose value fell within the range of + 1 to + 1.9 times the least significant difference for that character. Similarly the other classes contain means within the appropriate range limits. In this way all polycrosses in classes other than the zero class differ significantly from the average of the means of the three standard varieties for that character.

Comparisons of the polycrosses with the standard varieties may be made from the frequency distributions. For both fall growth vigour and stooling vigour, the polycrosses were, in general, clearly superior to the means of the three varieties. For general combining ability, these two measures of vigour show that the polycrosses were outstanding when compared with the average of the varieties. This leads to the conclusion that most of the seed produced in the polycross crossing plots was a result of cross-pollination.

Table 2.—Frequency distribution for agronomic characters of 21 polycrosses in Group I, and 22 polycrosses in Group II. Class centres are separated by multiples of the L.S.D. arranged ± on each side of the 0 class which is represented by the average of the three varieties in respective group

Character	Group	−3.5 or more	−2.5	−1.5	0	+1.5	+2.5	+3.5 or more	Total
Fall growth vigour	I	—	—	—	3	11	7	—	21
	II	—	—	—	—	—	2	20	22
Stooling vigour	I	—	—	—	2	3	6	10	21
	II	—	—	—	—	—	—	22	22
Date heading	I	—	3	8	10	—	—	—	21
	II	—	2	2	14	4	—	—	22
Height	I	—	—	6	14	1	—	—	21
	II	—	—	1	20	1	—	—	22
Weight per bushel	I	1	2	1	17	—	—	—	21
	II	—	2	6	14	—	—	—	22
Yield bu./acre	I	—	—	1	13	6	1	—	21
	II	—	—	—	7	13	2	—	22
Per cent fruitfulness	I	10	1	1	3	3	0	3	21
	II	11	2	0	3	2	1	3	22
Weight of 100 kernels	I	—	—	—	21	—	—	—	21
	II	—	—	3	19	—	—	—	22

Earliness, as indicated by date of heading, was exhibited by many of the polycrosses by comparison with the average performance of the standard varieties. A total of fifteen polycrosses headed out significantly earlier than the average of the varieties, while four polycrosses were significantly later. Twenty polycrosses matured significantly earlier and one significantly later than the average of the varieties.

The distribution of the polycrosses for height of plant was limited to a range of class centres 1.5 times the least significant difference on each side of the mean of the standard varieties. Altogether seven polycrosses were significantly shorter than the average of the varieties, while two were significantly taller.

The majority of the polycrosses did not differ significantly from the average of the varieties in weight per bushel. However, twelve had a significantly lower weight per bushel than the average of the varieties. Only one of the polycrosses had a significantly lower yield in bushels per acre than the mean of the varieties, while twenty-two were significantly superior in yield.

Comparisons may be made also in regard to the head and kernel characters studied. Per cent fruitfulness showed the widest range in variability of the characters studied. A total of twelve polycrosses significantly exceeded the average of the three varieties, while twenty-two had a significantly lower per cent of fruitfulness. Eighteen of those with a lower per cent of fruitfulness were more than four times the least significant difference below the average of the varieties. Many of the lines used in these polycrosses, as has been stated, originated from the Dakold variety. It will be noted that Dakold was lower in fruitfulness than either Emerald or Imperial in both groups of yield trials. When the polycrosses are compared directly with Dakold, the majority are equal to the parent variety or superior.

For weight of one hundred kernels the range in distribution about the mean of the varieties was extremely limited. Only three polycrosses differed significantly from the mean of the three varieties and for each of these the weight was lower than that of the varieties.

Pollen Sterility

The sterility determinations for 1947-48 were made on plants grown from remnant seed available from the polycross crossing plot. Sterility ranged from a low of two per cent for polycross 72 in 1947-48 to a high of sixty per cent for polycross 65 during the same season. The majority of the polycrosses were below fifteen per cent in pollen sterility. In order to determine the consistency of the pollen sterility estimations for the two years, the degree of association between the two estimations for those polycrosses grown in both years was calculated. The correlation coefficient for this association was + 901** for thirty-one polycrosses and was significant at the one per cent level. Realizing that the high sterility of Polycross 65 contributed disproportionately to this high coefficient, the degree of association was recalculated eliminating this polycross. The resulting coefficient, + 611**, was highly significant. Such a strong association indicated that the pollen sterility of the polycross was fairly similar in these two years and that the method of estimation was reasonably reliable.

Quartet Analysis

Twenty of the polycrosses were studied for the per cent of quartets showing micronuclei. Pollen sterility was determined from pollen of the same plants. A total of thirty-two plants were examined in this study. The numbers of quartets examined for each of two anthers per plant that were studied ranged from 81 to 401.

The per cent of micronuclei ranged from a low of zero for six plants to a high of 17.6 for polycross number 25 plant 4. The average per cent of abnormal quartets for all the plants examined was 1.35, while the average pollen sterility of these plants was 19.8. The sterility of the plants within a polycross was extremely variable, as also was the per cent of abnormal quartets. However, the variability in per cent of micronuclei between different anthers of the same plant was negligible in every case.

The degree of association between per cent of pollen sterility and per cent of quartets with micronuclei was calculated by means of the simple product moment correlation formula. In order to overcome the mathematical difficulties involving the use of zero categories, the data for per cent of abnormal nuclei were transformed to per cent normal quartets by use of the reciprocal. Both sets of percentage data were transformed to degrees of $\sin^2 \theta$. The association between per cent sterility and per cent normal quartets was of no importance or significance as shown by the correlation coefficient of − 0.066.

This low degree of association indicates that chromosomal abnormalities and difficulties of division resulting in micronuclei in the spore quartets are not a major cause of pollen sterility in these polycrosses. Presumably the cause of the pollen sterility must lie in some physiologic process under genic control.

Correlation Between Yield and Other Characters

Correlations between yield and other characters were studied by using the data in yield Groups I and II together. Before making these calculations a comparison was made for each of the characters studied including fall growth vigour; stooling vigour; date of heading; weight per bushel; weight per 100 kernels; per cent fruitfulness and yield for the three varieties grown in the two groups of yield trials which were made in adjacent areas. The comparisons were made by the t test. Except for the two characters yield and per cent fruitfulness there were no significant differences between the two yield trials for the average of these three varieties.

For yield the average of the three varieties in yield Group I was 58.6 bushels and in Group II, 48.7 bushels. The difference was highly significant. Percentage yield of each of the polycrosses in each group, therefore, was calculated in terms of the average yield of the three varieties taken as 100 when grown in the same group.

As the chief difference in per cent fruitfulness was due to a rather wide difference in reaction of the Imperial variety it was not considered necessary or desirable to correct this character. In these correlations the 43 polycrosses were studied as a group.

Simple product moment correlation coefficients were calculated for all interrelationships of the eight characters, fall growth vigour; stooling vigour; date of heading; weight per bushel; per cent fruitfulness, yield and weight per 100 kernels (see Table 3).

Several of the correlation coefficients were significant at the one per cent level, though all r values were less than ± 0.5. Yield was highly significantly positively associated with stooling vigour and with per cent fruitfulness. As might be expected, profuse stooling and a high per cent

TABLE 3.—SIMPLE PRODUCT MOMENT CORRELATION COEFFICIENTS FOR ALL INTERRELATIONSHIPS OF EIGHT CHARACTERS OF THE 43 POLYCROSSES OF RYE IN GROUPS I AND II

Character	A	B	C	D	E	F	G
	Yield	Fall growth vigour	Stooling vigour	Date of heading	Weight per bu.	Pollen sterility	Per cent fruitfulness
B Fall growth vigour	+.366*						
C Stooling vigour	+.448**	+.313*					
D Date of heading	+.274	+.374*	+.139				
E Wt. per bu.	+.186	−.183	−.283	−.314*			
F Pollen sterility	−.373*	−.384*	−.446**	−.301*	+.402**		
G Per cent fruitfulness	+.422**	+.023	+.223	+.245	−.144	−.263	
H Wt. per 100 kernels	+.017	−.261	−.143	+.006	+.051	+.169	+458**
D.F.	41	301*	389**				

of fruitfulness generally result in increased yields. Yield was also positively associated with fall growth vigour and negatively associated with pollen sterility, both significantly at the five per cent level. The association of yield and date of heading approached significance in a positive direction. Other associations with yield were extremely weak.

Fall growth vigour showed some significant associations with other characters, though none was highly significant. Fall growth vigour and stooling vigour were significantly positively associated, as were fall growth vigour and date of heading. The association of fall growth vigour and pollen sterility was significant in a negative direction.

Stooling vigour was highly significantly negatively correlated with pollen sterility. Polycrosses with poor stooling vigour might, therefore, be undesirable also due to high pollen sterility. Two other associations of stooling vigour, while not significant, may be of interest. The association of stooling vigour with weight per bushel approached the significant level in a negative direction, while that with per cent fruitfulness was positive

Date of heading was significantly negatively associated with both weight per bushel and pollen sterility at the five per cent level. Earlier polycrosses had a slight tendency to have higher weight per bushel and higher pollen sterility; however, many polycrosses could be expected to show a combination of the desirable characters. The association of date of heading and per cent fruitfulness approached significance in a positive direction.

TABLE 4.—SOME SIMPLE AND PARTIAL CORRELATION COEFFICIENTS OF EIGHT CHARACTERS OF RYE POLYCROSSES AND THE MULTIPLE CORRELATION COEFFICIENT OF YIELD WITH THE OTHER SEVEN CHARACTERS

Simple		Partial	
Characters associated	Correlation coefficient	Characters associated	Correlation coefficient
AC	+.488**	AC.BDEFGH	+.466**
AG	+.422**	AG.BCDEFH	+.374*
AF	−.373*	AF.BCDEGH	−.237
AB	+.366*	AB.CDEFGH	+.243
AD	+.274	AD.BCEFGH	+.233
AE	+.186	AE.BCDFGH	+.584**
AH	+.017	AH.BCDEFG	+.025
EC	−.283	EC.ABDFGH	−.373*
ED	−.314*	ED.ABCFGH	−.318*
EF	+.402**	EF.ABCDGH	+.349*
	R^2 A.BCDEFG .606		
	R .778**		

Code: A – Yield
B – Fall growth vigour
C – Stooling vigour
D – Date of heading
E – Weight per bu.
F – Pollen sterility
G – Per cent fruitfulness
H – Weight per 100 kernels

Weight per bushel and pollen sterility were highly significantly positively correlated. Thus polycrosses with a high weight per bushel had a slight tendency toward high sterility. Per cent fruitfulness and weight of one hundred kernels were highly significantly positively associated.

Relationship of all characters to at least one of the other characters used in the study was significant at the five per cent or higher level. Therefore, partial correlation coefficients were calculated using these simple correlation coefficients and are presented in Table 4. The appropriate simple correlation coefficients are presented also in this table for purposes of comparison.

The partial correlation coefficient for the association of yield with stooling vigour holding constant the effects of the other characters was highly significant. It is, however, only + 0.466 so that the character, stooling vigour, should be regarded merely as a fair indicator of yield. The association of yield with per cent fruitfulness, eliminating the influence of the other characters, was positively significant at the five per cent level. Per cent fruitfulness was a less reliable criterion of yield than stooling vigour. Both of these associations were closely similar to their respective simple correlation coefficients.

The partial correlation coefficient for the association of yield with per cent pollen sterility approached significance in a negative direction, though the simple correlation for these two characters was significant. The partial correlation coefficient for the association of yield and fall growth vigour and of yield and date of heading both approached significance in a positive direction.

The association of yield with weight per bushel was highly significantly positive when the other six characters were held constant, and was non-significant when these were not held constant. Apparently much of the association between yield and weight per bushel was obscured by the influence of other characters and eliminating this influence brought out the true relationship between yield and weight per bushel.

The only other significant partial correlation coefficients among the eight characters studied also involved the character weight per bushel. Weight per bushel was significantly negatively associated with stooling vigour, when the other six characters were held constant, and also significantly negatively associated with date of heading. The partial correlation coefficient for the association of weight per bushel and pollen sterility was significant positively.

The multiple correlation coefficient, indicating the dependence of yield on the total influence of the other seven characters, is also given in Table 3 and was highly significant. Slightly better than sixty per cent of the squared variability in yield was accounted for by the association with the other seven characters. While this value was highly significant, it still left a great deal of variation in yield unaccounted for by the influence of the characters studied.

The correlations, however, did make available some indication of the relative importance of the characters studied as criteria for the evaluation of the combining ability of the inbreds from their polycross performance.

The relative yield of the various polycrosses in comparison to the standard varieties might be expected to be of primary importance for this purpose. Stooling vigour appeared to be of some importance and, judging by the association with yield, of somewhat more value than per cent fruitfulness. Weight per bushel might be placed next in regard to possible value as an aid in selecting superior inbreds. Fall growth vigour and pollen sterility appeared equally low in value, since each showed about the same low degree of association with yield. Date of heading was probably of slight value and weight per hundred kernels of no value for predicting the possible yield performance of the inbreds in this study. While of no direct value in relation to yield, characters like weight of one hundred kernels and pollen sterility are of some interest in themselves.

Relation of Characters of Inbreds to Polycross Performance

Before making the selections of inbred lines to be recombined as synthetics, it appeared desirable to compare the polycross performance of the inbreds in relation to two characteristics not previously discussed in these studies. These were the length of time of inbreeding and the degree of self-fertility under isolation conditions.

Length of Time of Inbreeding

Data on performance in relation to the length of time of inbreeding are given in Table 5. The weight per hundred kernels appeared to show the effects of selection for plumpness, as there was a generally progressive increase in weight with increased number of years of self-pollination. On the other hand, the four long-time inbred lines had an average relative yield of 102 per cent, while the lines with four or five years' inbreeding had an average yield of 110 per cent; those with two or three years' inbreeding had a yield of 116 per cent, and the twenty-eight lines inbred only one generation, before being tested for combining ability, yielded 119 per cent. From the range in yield for the different groups, it is evident that long-continued self-pollination is unnecessary before testing for yield in polycrosses.

TABLE 5.—POLYCROSS PERFORMANCE OF INBRED SELECTIONS GROUPED ACCORDING TO THE NUMBER OF YEARS OF SELF-POLLINATION

Character	Years self-pollinated			
	1	2 and 3	4 and 5	8, 16 and 19
	Number of inbreds			
	28	6	5	4
Relative yield per cent	119	116	110	102
Range in yield per cent	96–139	104–125	81–131	99–108
Per cent fruitfulness	70	69	67	75
Fall growth vigour	4.1	4.7	4.2	4.0
Stooling vigour	4.3	4.4	3.7	4.0
Weight per bu.—lb.	55.8	56.6	57.4	55.5
Weight per 100 kernels—grams	2.60	2.60	2.80	2.85
Pollen sterility per cent	10.3	11.6	23.0	13.8

Degree of Self-fertility

Classification of the self-pollinated lines for self-fertility was attempted by studying data for the 1945 and 1946 crop seasons. The classification (see Table 6) was made on the basis of the number of plants in each line that were rather highly self-fruitful. Five classes were used: (1) all plants highly self-fertile; (2) most plants self-fertile with an occasional self-unfruitful plant; (3) approximately one-half of the plants highly self-fertile; (4) approximately one-fourth of the plants highly self-fertile; (5) all plants intermediate to low in self-fruitfulness.

In general, the differences in average relative polycross performance were negligible between groups. However, the highly self-fertile group was lowest in yielding ability with a range in relative yield for four lines tested in polycrosses of from 95 per cent to 111 per cent and a mean yield of 103 per cent.

Selection of Inbreds for Recombination as Synthetic Varieties

Having considered the relative value of the characters used in this study as indicators of yield or as of importance in themselves, the next problem is that of employing this information as an aid in selecting inbreds to be recombined as synthetic varieties. It has been mentioned that there were two classes of selections in separate polycrosses, i.e. colourless aleurone and green aleurone. Of those polycrosses tested in performance trials, thirty were progeny of colourless aleurone selections and thirteen were progeny of green selections. In the recombinations, these colour classes were maintained and the selection in the colourless aleurone group is discussed first.

In Table 7, the thirty colourless aleurone selections are listed in the order of their relative polycross yield performance on the basis of the average of the three varieties as 100 per cent. Weight per bushel, weight per hundred kernels, per cent fruitfulness and per cent pollen sterility are given also. Certain polycrosses greatly exceeded the varieties in yield. In selecting for recombination, polycross yield was considered of greatest importance and considerable attention was given to weight per bushel and per cent fruitfulness. Availability of inbred seed was also a factor in selection.

It was decided to combine as many lines as possible that were desirable in all characters making use of as much diversity of origin as was available. Therefore, thirteen inbred lines were combined in this group. These were 2, 3, 10, 25, 34, 37, 41, 50, 52, 55, 59, 61, and 64. These lines appeared

TABLE 6.—RELATIVE YIELDING ABILITY OF POLYCROSSES IN RELATION TO THE DEGREE OF SELF-FERTILITY OF THE INBRED LINES

Classification of inbred lines	No. lines	Range in yield	Mean yield
		per cent	per cent
Highly self-fertile	4	95–111	103.1
Mostly self-fertile	9	81–131	111.3
Half plants self-fertile	12	96–137	117.5
One-quarter plants self-fertile	11	109–139	122.6
Int. to low self-fertility	7	99–132	113.9

TABLE 7.—RELATIVE POLYCROSS PERFORMANCE OF COLOURLESS ALEURONE INBREDS OF RYE

Polycross line number or variety	Relative yield	Weight per bushel	Weight per 100 kernels	Per cent fruitfulness	Per cent pollen sterility
	per cent	lb.	g.		
59	139.4	56.7	2.8	75	10
55	137.2	56.1	2.7	75	10
21	134.9	57.2	2.6	67	15
48	132.7	55.3	2.6	77	10
10	130.6	58.0	2.7	71	20
52	130.0	55.8	2.9	74	10
58	127.9	54.9	2.5	80	5
53	126.1	54.4	2.8	68	5
41	125.9	57.3	2.5	74	15
56	125.7	56.6	2.4	72	5
12	125.1	57.9	2.6	71	15
64	124.6	55.6	2.8	71	10
24	123.6	54.5	2.6	69	5
29	123.2	55.6	2.6	61	10
25	122.8	56.6	2.7	68	10
61	121.4	54.7	2.8	75	10
50	120.7	55.7	2.9	78	20
31	117.2	55.5	2.6	73	5
37	116.6	55.8	2.8	67	10
34	116.0	55.7	2.7	72	15
2	110.8	57.5	3.0	73	15
17	110.6	54.8	2.6	72	10
22	110.5	57.0	2.3	61	15
35	109.2	53.9	2.3	70	10
3	105.1	56.2	3.2	83	15
8	101.0	57.3	2.8	65	20
30	100.2	55.1	2.4	61	10
4	98.8	52.5	2.7	72	10
51	95.7	53.7	2.6	67	10
6	95.4	56.2	2.8	65	20
Dakold	109.7	56.8	2.5	68	—
Emerald	102.5	56.3	2.8	74	—
Imperial	88.1	56.8	2.6	80	—

homozygous for colourless aleurone when the seed colour of the inbred lines was re-examined. The long-time inbred, highly self-fertile line 3 was included because, while it yielded only 5.1 per cent more than the average of the varieties, it excelled in seed size and was otherwise desirable. All other lines selected for this group were, in general, relatively satisfactory in all characters.

It seemed of interest also to combine a smaller number of lines selecting only those that excelled in polycross yield. For this group, six inbred lines were combined. The five highest yielders, lines 59, 55, 21, 48, and 10 with an index of yield performance above 130 per cent were selected. Inbred line 12 which had an index of yield of 125 per cent, and which excelled in weight per bushel was also selected, making three inbreds from Imperial and three from Dakold in this group.

For the green aleurone selections (see Table 8), six inbreds were combined in a group of the better lines and nine inbreds were combined in a second group. All lines were derived from Dakold. The six lines combined in the group of more desirable inbreds, when all characters were considered, were 67, 72, 73, 74, 76, and 78. Inbred 78 in the polycross yielded 107.5

per cent of the average yield of the three varieties, while the remaining lines ranged in yield from 11.6 per cent to 18.6 per cent more than the average of the varieties. The second group selected for combination consisted of the following nine lines: 13, 66, 67, 71, 73, 74, 76, 77, 78, and 81.

A final group of lower yielding lines was selected for recombination to serve as a basis for comparison of the performance of the other combinations. The lines selected for this group included colourless aleurone lines 4, 6, 8, 22, 30, 51 and the green aleurone, low yielding, line 65. Of these lines, 22 and 35 yielded in the polycross about the same as Dakold. The other lines ranged in polycross yield from 88.9 to 101 per cent of the average of the three varieties.

DISCUSSION OF RESULTS

Rye is generally considered to be largely cross-pollinated but, unlike corn, the greater percentage of the plants are rather highly self-sterile. However, the occurrence of more or less highly self-fertile plants is sufficiently frequent that selection within self-fertilized lines might be a reasonable method of breeding, provided, of course, that self-fertility is not, in itself, too undesirable as a plant character. In contrast to some other self-sterile, cross-pollinated plants, rye does not commonly fall into two classes of self-fertility, i.e. highly self-fertile or self-sterile, but commonly shows a wide range in the self-fertility of individual plants of the line in the early generations of self-pollination. Thus it is frequently possible to utilize selection as a means of raising the average self-fertility of a line over a period of years.

Combining highly self-fertile inbreds of rye has been of some value as a means of improving productivity. Such a practice would appear even more effective if lines were combined that had previously been shown to have good combining ability. The polycross method of determining combining ability appears well adapted for use with rye due to its mode of pollination.

TABLE 8.—RELATIVE POLYCROSS PERFORMANCE OF GREEN ALEURONE INBREDS OF RYE

Polycross line number or variety	Relative yield	Weight per bushel	Weight per 100 kernels	Per cent fruitfulness	Per cent pollen sterility
	per cent	lb.	g.		
13	119.6	57.3	2.4	63	10
72	118.6	57.3	2.6	74	10
73	117.9	57.0	2.5	66	15
67	117.6	57.0	2.8	68	10
74	115.9	56.8	2.7	70	15
76	111.6	56.5	2.5	62	15
66	108.2	57.3	2.7	73	20
78	107.5	56.3	2.5	66	10
81	106.8	56.7	2.5	64	5
71	104.4	55.1	2.9	68	10
77	103.1	56.2	2.5	69	10
75	99.3	55.6	2.5	63	5
65	80.9	57.1	2.7	57	50
Emerald	108.9	57.0	2.7	71	—
Dakold	102.4	57.3	2.5	69	—
Imperial	88.9	57.0	2.8	73	—

In this study, the polycross performance of forty-three inbred lines of rye was determined in replicated yield trials for several characters expressing combining ability. For many of these characters, significant differences were found between the polycrosses. However, correlation studies indicated that, of these, only vigour, weight per bushel and per cent of fruitfulness were of value as indicators of high yielding ability in the polycrosses. Weight per hundred kernels and weight per bushel were considered of some importance in themselves. Therefore, yield data, along with these other characters, appeared to be of greatest value in selecting lines to be recombined.

The performance of the polycrosses was studied in relation to two characters of the inbred lines. One of these was the length of time of inbreeding and the results obtained indicated that continued self-pollination and selection was of little value in the production of high yielding polycross progeny. In the material compared, the long-time self-pollinated lines yielded somewhat lower in polycrosses, on the average, than lines self-pollinated for only one generation. Lines self-pollinated for several generations yielded no better in polycrosses than lines self-pollinated for only a single generation. The other inbred character studied was the degree of self-fertility. The inbreds were classified into five groups, according to their self-fertility, ranging from all plants of a line highly self-fertile to all plants relatively low in self-fertility. Only one group differed noticeably in yield. The group classified as highly self-fertile, consisting of four lines that had been self-pollinated three years or more, was somewhat lower in yield, on an average, than the other groups. It appeared, therefore, that homozygosity for high self-fertility was not an advantage in the production of high yielding polycrosses.

These two characters have been studied in alfalfa also. Alfalfa breeding, as practised by several prominent workers, was based to a large extent on selection within inbred lines. Current practice appears more concerned with the isolation of high combining clones without resorting to inbreeding, except when desirable in relation to some particular character. Where combining ability was determined by the polycross method, it has been suggested that high self-fertility was not a desirable character for the clones being combined. High self-fertility may lead to the production of considerable self-pollinated seed in the seed plot, which may produce progeny lacking in hybrid vigour when grown in comparison with cross-pollinated seed.

The polycross has been used in alfalfa breeding as an aid in selecting high combining clones and as a means of combining these selected clones. In a comparison of the polycross method with average top cross and single cross performance, Tysdal and Crandall (5) found that the polycross was as good as either of the othei methods as an indicator of the relative combining ability of eight clones. The synthetic variety resulting from recombining clones selected by this method was significantly higher than the standard variety in yield and several other characters.

It appears logical to expect satisfactory results in rye breeding since, in these studies, many of the polycross progenies gave excellent yields in comparison with the standard rye varieties. Apparently there must have been a high degree of cross-pollination in the polycross plots even though the parent plants were selected for high seed production when self-pollinated.

SUMMARY

1. Selection in self-pollinated lines of rye has been practised in a rye improvement study in Minnesota. Individual plants were selected that excelled in seed-setting under isolation, in seed characters and also in vigour. While several lines had been self-pollinated for many years, a considerable proportion had been self-pollinated for only one generation. The majority of the lines were derived from the varieties Dakold and Imperial, though other sources were also represented.

2. Sixty-four colourless aleurone selections of rye that had been self-pollinated one to nineteen generations were grown in an isolated polycross nursery, allowed to interpollinate at random and seed was bulked from all the individual plants of a selection. Twenty-three green aleurone selections, self-pollinated one to eight years, were grown and handled similarly in another isolated polycross nursery.

3. Polycrosses of thirty of the colourless aleurone selections and thirteen of the green selections were selected and grown the following year in replicated yield trials. Group I of the yield trials consisted of twenty-one polycrosses, while Group II consisted of twenty-two polycrosses. The varieties Dakold, Emerald and Imperial were included with each group.

4. Average performance data were reported for each polycross and variety for yield, fall growth vigour, stooling vigour, date of heading, height of plant, weight per bushel, per cent fruitfulness, weight per 100 kernels, and pollen sterility.

5. Analyses of variance indicated that significant differences existed between the polycrosses for each of these characters.

6. In comparison with the standard varieties most of the polycrosses were significantly superior in yield and vigour.

7. Correlation coefficients for the 43 polycrosses as one group were determined for all interrelationships of the characters yield, fall growth vigour, stooling vigour, date of heading, per cent fruitfulness, weight per bushel, pollen sterility, and weight per hundred kernels. Highly significant associations were found between yield with stooling vigour and per cent fruitfulness, between weight per bushel and pollen sterility, between per cent fruitfulness and weight per 100 kernels and negatively between stooling vigour and pollen sterility. Several other associations were significant at the 5 per cent level.

8. Significant partial correlations were found for the association of yield with stooling vigour, weight per bushel, and per cent fruitfulness, eliminating the influence of the other characters in each case.

9. Slightly better than 60 per cent of the squared variability in yield was accounted for by its association with six characters as determined by the multiple correlation.

10. Length of time of inbreeding and degree of self-fertility under isolation were studied in relation to polycross performance. Selection during inbreeding appeared effective in increasing seed size. Selected plants that produced a large number of desirable seeds the first year of self-pollination yielded, in polycrosses, on the average, as well as, or slightly better, than polycrosses from lines self-pollinated from two to five years.

Four inbred lines self-pollinated eight to nineteen years ranged in yield from 99 to 108 per cent of the average of the varieties and were lower in average yield than groups self-pollinated for a shorter period.

The lines studied in polycrosses were placed in five groups for self-fertility as follows: (1) all plants highly self-fertile; (2) most plants self-fertile with an occasional self-unfruitful plant; (3) approximately one-half of the plants highly self-fertile; (4) approximately one-fourth of the plants highly self-fertile; (5) all plants intermediate to low in self-fruitfulness. In general, the groups yielded about the same, although the highly self-fertile group had the lowest average yield.

11. In selecting inbreds for recombination on the basis of combining ability, it appeared that yield of the polycross progeny should be given first consideration. Stooling vigour, per cent fruitfulness, and weight per bushel were of some importance. Other characters though of importance in themselves were not useful as indicators of yield.

12. Five recombinations were set up to test the value of the selections made on the basis of polycross performance.

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STUDIES ON STRONGLY ALKALINE SOILS OF THE SALT RIVER PLAINS IN NORTHWESTERN CANADA[1]

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During the chemical examination of a number of soil samples collected in 1945 from Northwestern Canada, it was observed that certain subsurface samples from the Salt River plains (Figure 1) had extremely high pH values (up to 9.6) but contained only small amounts of water-soluble salts. Such areas supported a very good growth of grass and trees (Figures 2 and 3). On the other hand, certain other samples from barren areas (Figure 4) in the vicinity contained considerably higher concentrations of soluble salts and their reaction did not exceed pH 8.5. It was therefore considered that a more complete study of these soil samples would be of interest.

The area from which the samples were collected lies adjacent to the Salt River about 20 miles southwest of Fort Smith, N.W.T. The river here flows through a level plain of lacustrine and alluvial origin. The area is noted locally for the brine springs which occur at the base of a prominent escarpment marking the western limits of the plain, and for the presence of large flats barren of vegetation.

The four profiles (Table 1) studied in the investigation were selected as being typical of the major mineral soils of the area which form an interesting combination of soils developed under trees, under grass, and without any natural vegetation. Profile D is a saline soil; profile C is an immature chernosem; profile G is a grey wooded soil; profile I is a degraded chernosem.

LABORATORY DETERMINATIONS

In Table 1 are presented the results of certain chemical determinations on the soil samples. These determinations include reaction, nitrogen, calcium carbonate, and content of water-soluble material together with estimated amounts of sodium chloride and sodium carbonate therein. The pH values were determined by means of the glass electrode and nitrogen by a modification of the Kjeldahl method. The CO_2 liberated by treatment with hydrochloric acid was calculated as $CaCO_3$. The water soluble material was obtained by shaking the soil with two and one-half times its weight of water, letting stand over night and passing the extract through a Pasteur-Chamberland filter candle. The filtrates were analysed for calcium, magnesium, potassium, sodium, chloride and sulphate, and the probable amounts of NaCl and Na_2CO_3 were calculated from these results.

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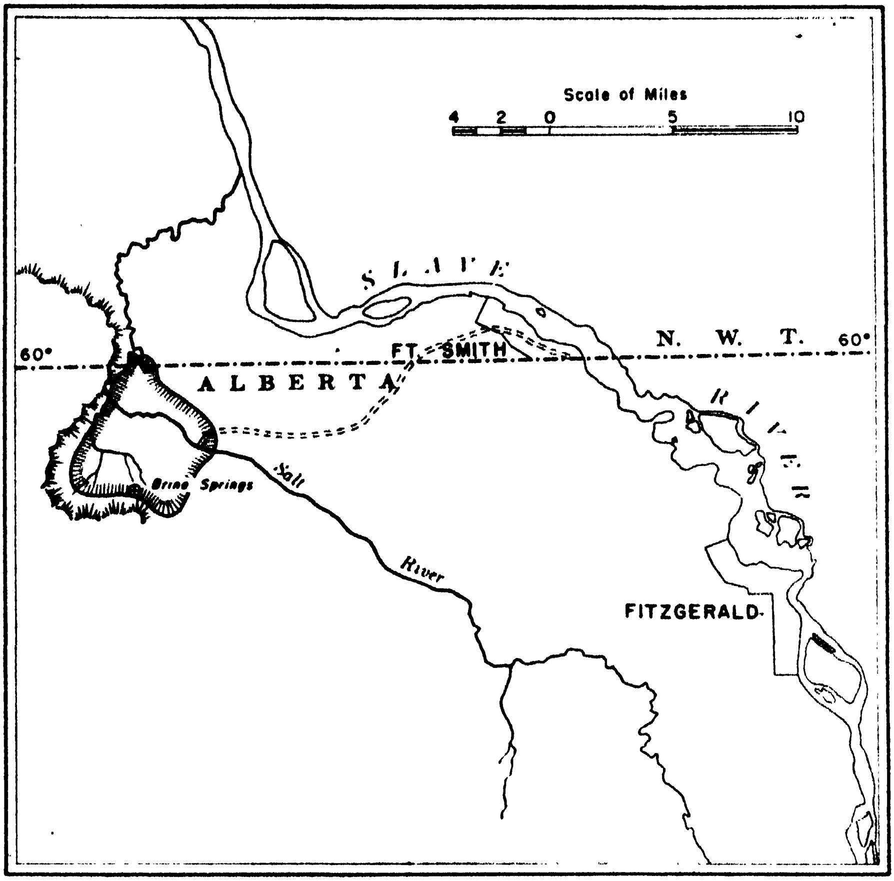

FIGURE 1.—Map showing Salt River and location of area sampled.

$CaCO_3$ and pH

There appeared to be no relationship between soil reaction and the amount of calcium carbonate present. In profile D, the $CaCO_3$ content ranged from 68 to 27 per cent and the reaction was pH 8.3. In the other profiles where the reaction was pH 8.9 or above, the $CaCO_3$ content did not exceed 20 per cent. Buehrer and Williams (2) in Arizona showed the absence of any correlation between percentage of $CaCO_3$ in soils and their pH values.

Soluble Salts

In profile D where there was little or no vegetation, the water soluble material after ignition averaged over two per cent of the dry soil. At the other locations where there was considerable growth of trees or grass, the amounts of water-soluble constituents were much less. It is worthy of note that the main constituent of the solids extracted from the high salt area was sodium chloride and made up approximately 80 to 90 per cent of the ignited material. Such high concentrations of the chloride ion in Canadian soils is rather unusual. In a study of alkali soils in Saskatchewan,

FIGURE 2.—Area supporting good growth of grass.

Mitchell (7) stated that "the soluble salts present are commonly sulphates of calcium, magnesium and sodium, the chlorides being relatively uncommon." This has been corroborated by other workers (3, 5, 8).

The sodium that could not be accounted for as chlorides and sulphates was calculated as Na_2CO_3. In the extracts from profile D, the sodium that was estimated to be in the carbonate form did not exceed five per cent of the ignited material and the reaction of the soil samples was in every case less than pH 8.5. In profiles C, G and I where samples taken at greater

TABLE 1.—ANALYSIS OF SOIL SAMPLES FROM SELECTED PROFILES

Profile	Vegetation	Depth of sample	N	$CaCO_3$*	pH	Total solids on ignition		
						as per cent of soil	NaCl content	Na_2CO_3 content
		(in.)	%	%			%	%
D	None	0- 6	0.07	67.97	8.3	2.10	89	5
		6-12	0.05	44.78	8.3	1.77	92	3
		12-18	0.04	27.60	8.3	2.48	90	2
C	True upland grassland	0- 6	0.25	—	6.6	0.38	88	7
		6-12	0.14	29.02	8.6	0.63	85	14
		12-18	0.15	19.76	9.3	0.45	—	—
		18-24	0.13	17.59	9.0	0.38	90	13
G	Large poplars	0- 2	1.90	—	6.2	0.09	28	0
		2- 4	0.27	—	5.8	0.06	63	18
		4- 8	0.08	—	6.2	0.03	—	—
		8-12	0.09	1.17	7.7	0.04	—	—
		12-18	0.06	16.76	9.2	0.08	57	47
		18-27	0.05	17.43	9.6	0.10	70	35
I	Prairie	0- 6	0.28	—	6.7	—	—	—
		6-12	0.07	13.93	9.1	0.29	74	22
		12-18	0.06	13.01	8.9	0.23	79	20
		18-24	0.05	9.76	9.0	0.23	77	20

* Calculated from CO_2 liberated by HCl.

TABLE 2.—pH AND EXCHANGEABLE BASES IN THREE PROFILES

(As milliequivalents per 100 gm. air-dry soil)

Profile	Vegetation	Depth of sample	Exchangeable bases				pH
			Ca	Mg	K	Na	
		(in.)					
C	True upland grassland	6–12	15.8	4.4	0.3	18.1	8.6
		12–18	12.4	3.8	0.2	15.7	9.3
		18–24	10.8	2.9	0.2	13.9	9.0
G	Large poplars	4–8	10.0	3.3	0.2	1.0	6.2
		8–12	18.1	5.4	0.2	1.1	7.7
		12–18	11.6	4.5	0.2	3.7	9.2
		18–27	10.4	4.8	0.1	4.7	9.6
I	Prairie degraded appearance	6–12	9.1	3.6	0.2	10.2	9.1
		12–18	8.7	3.4	0.2	10.5	8.9
		18–24	7.8	3.0	0.1	9.3	9.0

TABLE 3.—EFFECT OF HOT WATER EXTRACTION ON REACTION AND SOLUBLE MATERIAL

Type of extraction	pH of soil		Total solids on ignition		
	Before extraction	After extraction	as per cent of soil	NaCl content	Na_2CO_3 content
				%	%
Profile G					
12 to 18 inches					
Cold water	9.3	9.2	0.16	66	22
Hot water	9.3	8.6	0.47	50	27
Profile I					
18 to 24 inches					
Cold water	9.2	9.6	0.46	68	20
Hot water	9.2	8.6	0.75	54	27

depth (down to 24 and 27 inches) were available, reactions as high as pH 9.6 were found. In these cases, the estimated content of sodium carbonate was usually considerably in excess of ten per cent of the ignited material. Because of the low quantities of soluble salts in these profiles, however, the total amounts of sodium carbonate present would be about the same as in the samples of profile D.

Exchangeable Bases

Samples from profiles C, G and I which had pH values above 8.5 were submitted to more thorough examination. Exchangeable bases were determined by extraction with neutral normal ammonium acetate solution. It was found that large amounts of sodium were present, in most cases exceeding the amounts of exchangeable calcium, as shown in Table 2. Although in profile G, the two samples (12 to 18 inches and 18 to 27 inches) with pH values above 9 had less exchangeable sodium than calcium, the proportion of sodium was much greater, and that of calcium somewhat less, than in the 8 to 12 inch sample whose pH value was 7.7.

FIGURE 3.—Area supporting good growth of trees.

Water Extraction and pH

Samples of these three profiles were also extracted and leached with approximately ten times their weight of cold, distilled water. Although the extracts were found to contain very large amounts of sodium, mostly as chloride but with a considerable quantity as carbonate, the pH values of the soil samples themselves were not reduced to any extent by the extraction. In fact, in some cases, the pH values of the extracted soil were higher than those of the original samples.

In view of the fact that sodium carbonate (Na_2CO_3) is considerably more soluble in hot water (48.5 per cent at 104° C.) than in cold (7.1 per cent at 0° C.) (4), it was decided to determine what effect extraction with hot water would have on the pH values of these soils and on the composition of the extracted material. Accordingly, two samples (profile G, 12 to 18 inches and profile I, 18 to 24 inches) were extracted by placing soil and

TABLE 4.—EFFECT OF LEACHING ON REACTION OF SOILS CONTAINING $CaCO_3$ AND NaCl

Soil	Treatment	pH values	
		Before leaching	After leaching
No. 1	Untreated	7.0	7.4
	$CaCO_3$	7.8	8.5
	$CaCO_3$ + NaCl	7.7	9.1
No. 2	Untreated	8.1	8.6
	NaCl	8.0	9.6

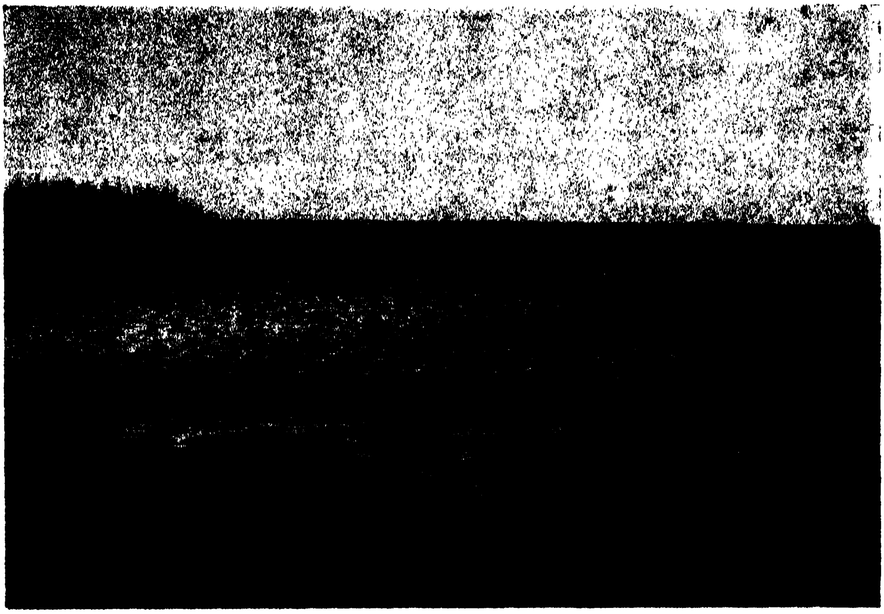

FIGURE 4.—Barren area with salt incrustations.

distilled water in bottles in a hot water bath for several hours, then removing the supernatant liquid and filtering it. This method of extraction was repeated several times, following which the residues were air-dried and their pH values determined. The various successive hot water extracts were combined and analysed. The comparative results of hot and cold water extractions for these samples are given in Table 3. In both cases, extraction with hot water brought about decreases in pH values. Considerably more material was extracted with the hot water and this contained a greater proportion of sodium as the carbonate, less as the chloride.

NaCl, $CaCO_3$ and pH

It was believed that, if the high pH values of these soils were due to the presence of sodium carbonate, this might have been formed by a reaction between calcium carbonate in the soil and sodium chloride from the brine springs which, as mentioned in the introduction, are situated not far from where the samples were taken. In order to determine the effect of NaCl and $CaCO_3$ on soil reaction, a laboratory experiment was conducted on two soils, one of which was neutral in reaction (pH 7.0) whereas the other was strongly alkaline (pH 8.1) and contained much free carbonate as shown by strong effervescence when treated with hydrochloric acid.

The neutral soil (No. 1) was treated with both calcium carbonate and sodium chloride (20 gm. $CaCO_3$ and 4 gm. NaCl to 400 gm. soil). The alkaline soil (No. 2) was treated with sodium chloride only, at the rate used on soil No. 1. These, together with untreated samples, were alternately moistened and dried over a period of six months, at the end of which time, all samples were tested for reaction, then leached with several times their weight of cold, distilled water and again tested for reaction. The results obtained are presented in Table 4.

In soil No. 1, the addition of $CaCO_3$ increased the pH value as expected. In both soils, where NaCl was introduced in the presence of $CaCO_3$, the reaction of the samples before leaching was the same as where NaCl was omitted. In all cases, leaching brought about some increase in pH values. However, the reactions of the NaCl-treated soils were increased to a considerably greater extent and values above pH 9 were obtained.

DISCUSSION

Probably the most useful single determination made on soil samples is that of reaction (9). As mentioned above, Buehrer and Williams (2) showed the absence of any correlation between percentage of $CaCO_3$ in soils and their pH values. Bailey (1) pointed out that pH values higher than 8.5 usually reflect the presence of Na_2CO_3 or other alkaline salt accumulated under poor drainage conditions. McGeorge (6) stated that "among the variables affecting pH are salinity, both concentration and character, and the anions and cations as individuals, the character of the base exchange complex, and the mechanical composition of the soil."

In McGeorge's work, the failure to find any correlation between pH and total salts in the soil was "explained by the observation that HCO_3 and CO_3 ions have opposite effects on pH to the Cl and SO_4 ions . . . . one should not necessarily expect any relation between total salinity and pH, nor between sodium and pH. The salinity effect on pH appears to be largely due to the anions." McGeorge also observed that "the pH of the soil increases with increase in percentage of replaceable sodium and the ratio of sodium to the total exchange capacity of the soil."

SUMMARY

The results obtained in the investigation of soil samples from the Salt River plains in Northwestern Canada would appear to agree fairly well with those to which reference has just been made. Where the sodium chloride concentration was found to be high (85 to 90 per cent of the total soluble material after ignition) the soil reaction seldom exceeded pH 8.5 although appreciable amounts of sodium carbonate were present. On the other hand, where the soil reaction was in the vicinity of pH 9.0 or higher, the amount of sodium carbonate present was approximately the same as in the samples with lower pH values but the total amount of soluble salts was much less. Thus the ratio of sodium carbonate to the other soluble salts present, rather than the actual amount of sodium carbonate, was indicated to be an important factor in determining the pH value of the soil. In the laboratory experiment where NaCl was mixed with soil in the presence of $CaCO_3$, no change in pH value was obtained until the soil was leached. It would appear, therefore, that the high pH values of the soil samples under investigation were due to the presence of sodium carbonate which could have been formed by the interaction of sodium chloride and calcium carbonate in the soil, followed by leaching.

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A NEW TYPE OF BACTERIAL SPOILAGE IN CANADIAN PROCESS CHEESE[1]

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To what extent Canadian manufacturers of process cheese have encountered bacterial spoilage of economic importance is not definitely known. Outbreaks involving two widely separated cheese firms have been drawn to the attention of the Division during the past year. In the first instance reported, the defect was confined to half-pound retail packages of process cheese, while in the second outbreak only the 5-pound process cheese showed spoilage. As the trouble had not been experienced previously in these two plants and had proved to be a costly defect, an explanation was sought as to its cause and method of control.

Griffiths (1) reports instances of spoilage of process cheese by *Clostridium coagulans* which involved a bleaching of the colour, the development of softened and crumbled areas in the cheese, and a fecal odour. In some of the affected areas a small dark slit was distinguishable which was thought to be caused by the formation of gas. The source of the infection was not determined. Other investigators (2) have isolated *Clostridium sporogenes* and *Clostridium welchii* from defective samples of tinned process cheese. In the cases reported, the cheese had undergone a gassy fermentation with the development of a putrid odour. The use of skimmilk powder which contained 2 per cent of lactose was believed to have had a bearing on the development of the defect as the putrefactive condition was observed only in cheese to which skimmilk powder was added. The same types of organisms were isolated from cheese which contained no milk powder and thus no additional lactose, but in no instance did the organisms develop and cause deterioration. The authors conclude that the exact part played by the lactose in its association with the defect was not clearly established. Csiszar (3) found that spores of *Clostridium sporogenes* were destroyed only by temperatures which were too high to make a satisfactory process cheese.

NATURE OF THE DEFECT

The samples of defective process cheese submitted by both manufacturing firms were similar in appearance and of identical odour on examination. In all cases, the cheeses were badly swelled or puffed, contained numerous gas-holes in the centre of the cheese, and were accompanied by a very obnoxious, penetrating, putrefactive odour. Some variation was found in the degree of swelling or bulging, apparently due to the age of the cheese or to retail temperatures of storing and handling, or to the initial amount of the infection. All samples submitted for examination were definitely inedible.

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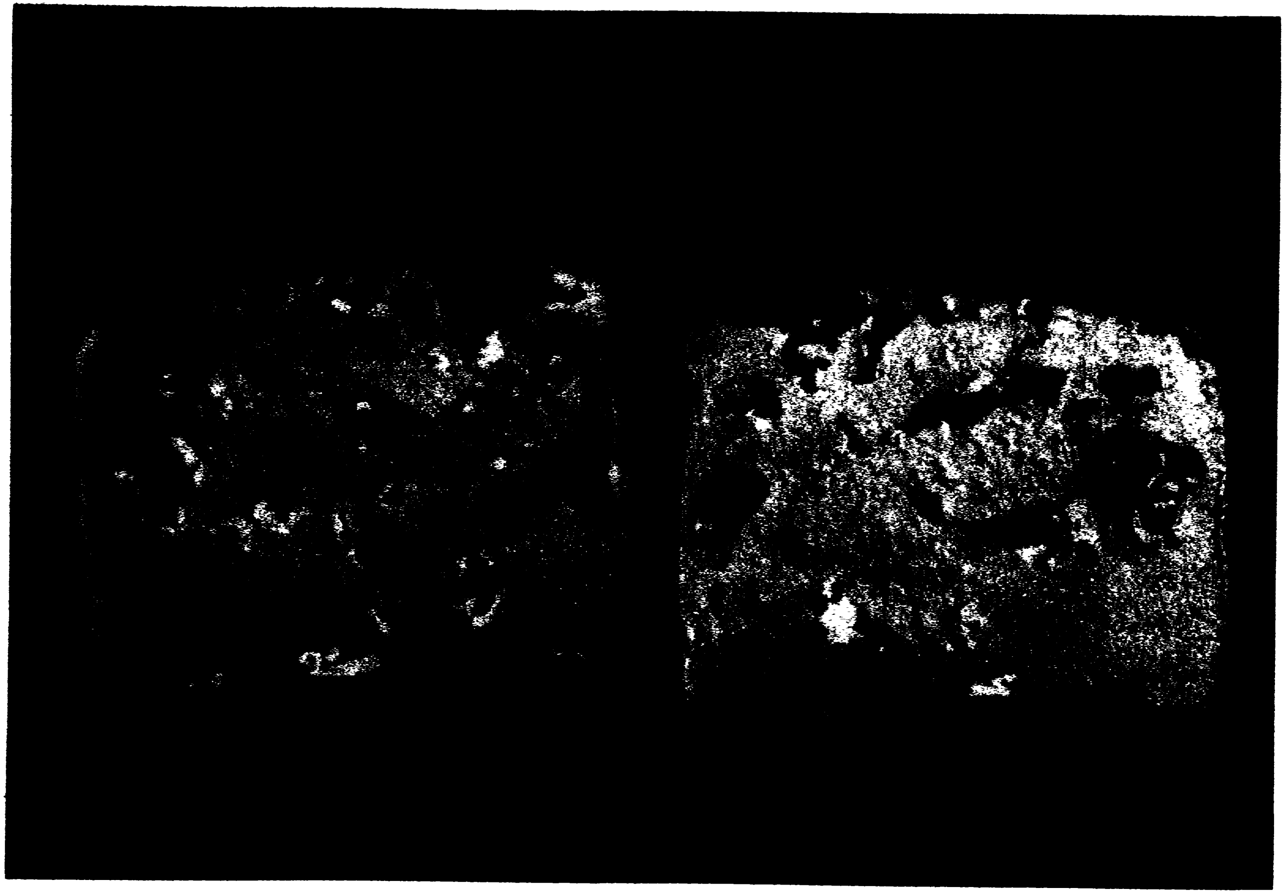

FIGURE 1. Appearance of the defect in a half-pound package of process cheese.

OCCURRENCE

According to information supplied by the manufacturers, the defect is much more prevalent in summer than in winter and has been found in plant control check samples as well as in the retail trade. While the occurrence of the defect is usually occasional, it has been found in all batches of cheese made on the same day. Control test packages held at 100° F. for observation by the manufacturer showed bulging in 24 to 48 hours, while in other instances 3 to 4 days were required. Comparative test packages held at summer temperature developed the defect more slowly than at 100° F. but eventually showed bulging in 1 to 2 weeks.

PROCESSING

Both manufacturing plants report that standard processing procedures were in use when the trouble was encountered. Temperatures for the effective pasteurization of the cheese from a public health standpoint were used in all cases. Infection from plant equipment was ruled out as a possible source of the cause of the defect. It was, however, revealed that a change had been made in the process cheese mix and that skimmilk powder had been substituted in varying percentages (from 2 to 10) to replace other milk solids. It was also stated by one manufacturer with many years of successful operation that the defect coincided with the introduction of skimmilk powder, and particularly so where high percentages were used. It was also demonstrated by the same manufacturer that the defect did not develop in process cheese with the old formulae which did not call for skimmilk powder, nor did it develop in the half-pound packages containing 2.5 per cent of skimmilk powder.

ISOLATION AND IDENTIFICATION OF ORGANISM

Microscopic examination of the defective cheese showed the presence of large numbers of spores and spore-forming bacteria resembling Clostridia. Tubes of thioglycollate medium + 0.5 per cent starch were inoculated with samples of the cheese and incubated at 37° C. Growth was obtained in 3 days. The cultures were purified by plating on Spray's liver veal agar and by picking colonies into thioglycollate medium. A description of the cultural and biochemical characteristics of the organism follows:

> Motile, Gram positive, straight rod with rounded ends, occurring singly, in pairs, or less frequently in short to long chains.
>
> Spores oval, sub-terminal with swelling rods.
>
> Growth on agar slant, spreading, rhizoid and opaque.
>
> Agar colonies woolly with dense centre.
>
> Produces a turbid, flocculent sediment and putrid odour in nutrient broth.
>
> Rapidly digests litmus milk.
>
> Does not blacken brain medium or coagulated albumen. Hydrogen sulphide is produced, but nitrates are not reduced nor indol formed.
>
> Causes fermentation of dextrose and maltose with formation of gas, but does not attack lactose or sucrose.
>
> The organism is anaerobic with an optimum temperature of 37° C

On the basis of these tests, and tests conducted by the Laboratory of Hygiene, Department of National Health and Welfare, the organism was identified as a variant of *Clostridium sporogenes* (4).

MAIN SOURCES OF INFECTION

The main source of the organism causing spoilage has been found to be associated with certain batches of skimmilk powder used in the manufacture of process cheese. While it has not been possible to isolate *Cl. sporogenes* from all samples of skimmilk powder, it has, however, been found in 50 per cent of the samples examined. The organism has also been reported to be present in raw milk Cheddar cheese by one of the commercial plants experiencing trouble. An examination of the sample submitted to our laboratory by this company gave negative results. In the absence of supporting data, it is suspected that some vats of Cheddar cheese may contain the organism. Whereas the source of infection has been definitely traced to skimmilk powder, the main factor in initiating spoilage appears to be related more to its content of available nutrients for the growth of *Cl. sporogenes.*

EXPERIMENTAL

Table 1 shows the effect on experimental batches of process cheese made with the addition of different milk solids when uninoculated and inoculated with spores of *Cl. sporogenes.*

TABLE 1.—EFFECT OF *Cl. sporogenes* ON EXPERIMENTAL PROCESS CHEESE CONTAINING MILK SOLIDS IN THE FORM OF SKIMMILK POWDER, CASEIN DIGEST AND AGED CHEESE

Main ingredients (1)	No. of spores added per gram of process cheese		
	0	1000	5000
4-Months old Cheddar cheese	Normal cheese	Normal cheese	Normal cheese
As above + 2.5 per cent sterile skim powder	Normal cheese	Slight bleaching	+++
As above + 5.0 per cent sterile skim powder	Normal cheese	+ (3)	+++
As above + 10.0 per cent sterile skim powder	Normal cheese	++	+++
As above + 2.0 per cent casein digest (2)	Normal cheese	++	+++
1-Year old Cheddar cheese	Normal cheese	+++	+++

(1) = Experimental batches of process cheese held for 5 days at 98° F.
(2) = N-Z- Case. Sheffield brand of tryptic digested casein.
(3) = + - Definite gas.
++ - Pronounced gas and proteolysis.
+++ - Very pronounced gas and proteolysis.

DISCUSSION

It will be noted in Table 1, column 2, that all of the experimental control batches of process cheese which were free of *Cl. sporogenes*, but which contained different percentages of sterile skimmilk powder and casein digest, remained normal when incubated at 98° F. for 5 days. Process cheese made from raw Cheddar of 4 months' age but containing no added milk constituents in the form of skimmilk powder or casein digest, remained normal even when inoculated with 5000 *Cl. sporogenes* cells per gram of cheese. Process cheese made to contain 2.5 per cent of sterile skimmilk powder showed only a slight trace of bleaching with no evidence of spoilage when inoculated with 1000 spores per gram of cheese but very marked spoilage with 5000 spores per gram. With the addition of 5.0 and 10.0 per cent of sterile skimmilk powder and 2.0 per cent of casein digest all experimental batches were badly blown and accompanied by a very strong putrefactive odour. Spoilage was more pronounced with the higher inoculum. Process cheese made from one-year old Cheddar cheese but without the addition of skimmilk powder when inoculated showed as marked spoilage as did the process cheese made with the addition of varying percentages of skimmilk powder and the 2.0 per cent casein digest.

It is evident from the data submitted that two factors were involved in spoilage, namely, the load or number of organisms present in the process cheese at the time of manufacture, and secondly, the presence of nutrients which were supplied by the addition of sterile skimmilk powder, casein digest, or from mature Cheddar cheese. The latter factor, however, is much more important than the former, as it has been found impossible to initiate spoilage in highly infected experimental process cheese when no nutrients were added in the form of milk solids. It follows, therefore, that the greater the percentage of skimmilk powder added, the more rapidly will spoilage develop.

While no supporting data are available, it is suspected that some vats of Cheddar cheese may contain the organism in sufficient numbers to cause spoilage if the proper nutrients are provided in the process cheese by the addition of skimmilk powder or by the use of high percentages of mature Cheddar cheese.

PRACTICAL IMPLICATIONS

According to manufacturers, skimmilk powder is an essential and desirable ingredient in the making of process cheese. As this product has been found to be the major source of infection as well as providing nutrients for *Cl. sporogenes* in process cheese, it would appear highly desirable to have all powders used in the manufacture of process cheese examined by special bacteriological methods.

SUMMARY

1. Two widely separated outbreaks of bacterial spoilage in process cheese reported to the Division have been investigated.

2. An organism whose characteristics correspond closely to those of *Clostridium sporogenes* was found to be the causal organism responsible for the defect.

3. The organism has been isolated from defective process cheese and from 50 per cent of the samples of skimmilk powder examined.

4. The defect was not reproduced in experimental batches of process cheese made from four-months-old Cheddar cheese even when heavily inoculated with the organism.

5. The defect was readily reproduced in experimental batches of process cheese made with different percentages of skimmilk powder, with 2.0 per cent casein digest, and with one-year-old Cheddar cheese and inoculated with *Cl. sporogenes.*

6. In addition to being the main source of infection, skimmilk powder furnishes the necessary nutrients for the development of the organism in process cheese.

7. As the number of organisms added to process cheese hastens the development of spoilage, the greater the percentage of infected skimmilk powder used the greater is the amount of infection added.

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THE AMINO ACID COMPOSITION OF THE HORSEBEAN (*VICIA FABA* L.) AS RELATED TO ITS NUTRITIVE VALUE FOR CHICKS[1]

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INTRODUCTION

Canadian crops yielding a supply of protein meal suitable for chickens are relatively few. The horsebean, however, thrives in the lower St. Lawrence area and the Lake St. John area of Quebec Province. Nickolaiczuk, Brisson and Mani (9) have studied the use of horsebeans as a source of vegetable protein for growing chicks. The results showed that the principal amino acid deficiency of horsebean proteins is its low methionine content, and that horsebean protein fortified with this amino acid might be of considerable value as a protein for chicks.

Information on the amino acid composition of horsebean protein appears to be limited. It was decided, therefore, to conduct microbiological assays of its amino acid composition. The results of this study are now presented and discussed in relation to the findings of Nickolaiczuk *et al.* (9).

MATERIAL STUDIED

The sample of horsebeans studied was typical of those grown in Quebec Province. It contained 27.6 per cent crude protein.

PREPARATION OF HYDROLYSATES FOR MICROBIOLOGICAL ASSAY

The whole beans were finely ground and dried to constant weight *in vacuo* at 80° C. Acid hydrolysates were prepared directly from this material by sealing weighed samples in glass tubes with 10 ml. of 10 per cent hydrochloric acid (2.5 N) per gram of meal. The sealed tubes were then autoclaved for 10 hours at 121° C. The cooled hydrolysates were filtered, and any precipitate of humin was broken up and thoroughly washed with distilled water. Some sodium acetate was added and the acidity adjusted to pH 4.0. The hydrolysates were then made up to a suitable volume, covered with a layer of toluene and stored at approximately 5° C. pending assay.

In the case of cystine assays the hydrolysate was prepared from a protein fraction obtained as described below instead of by direct hydrolysis of the ground beans.

Alkaline hydrolysates were used exclusively in the cases of assays for tryptophane and tyrosine. The hydrolysates were prepared in the same manner as the acid hydrolysates, but using 10 ml. 20 per cent sodium hydroxide per gram of material instead of hydrochloric acid.

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EXPERIMENTS ON THE EXTRACTION OF A PROTEIN FRACTION

The large proportion of carbohydrate in the beans leads to heavy losses of cystine if the hydrolysis is carried out directly. Experiments were made, therefore, in order to determine optimal conditions for the extraction of the protein from the ground beans by dilute alkali. Preliminary experiments showed that it was necessary to grind finely in order to obtain satisfactory extraction. Accordingly "fine bean meal" samples were prepared by further grinding the meal for 48 hours at 120 revolutions per minute in a small ball mill. A weighed portion was taken in a centrifuge tube and shaken vigorously for 15 minutes with 50 ml. of 0.08 per cent sodium hydroxide for 15 minutes. The mixture was centrifuged and the clear supernatant was decanted. Two further similar extractions of the residue were carried out. The united clear brown extracts contained 94.3 per cent of the total nitrogen of the fine meal. Further extractions did not appreciably increase the proportion of nitrogen extracted.

A series of similar extractions was carried out using increasing strengths of sodium hydroxide up to 0.40 per cent. Increasing the strength of the alkali resulted in a slightly greater extraction of the total nitrogen, but this trend could not be put to practical use. As the concentration of sodium hydroxide exceeded 0.10 per cent, marked swelling of the residue took place with a tendency to form colloidal suspensions. In order to avoid this difficulty and to secure as complete separation of the protein from the carbohydrate material as possible, it was decided to adhere to the use of 0.08 per cent sodium hydroxide as extractant.

The optimal pH for the precipitation of the protein from the extract was determined by extracting a number of 1.0 gm. samples of fine meal with 0.08 per cent caustic soda as described above. The extracts were collected separately and adjusted to acidities ranging from pH 3.4 to pH 4.4. The resultant precipitates were separated by centrifuging and analysed for nitrogen. The results are set out in Table 1.

TABLE 1.—EFFECT OF pH UPON PRECIPITATION OF PROTEIN FROM ALKALINE EXTRACTS OF HORSEBEAN MEAL

pH	Nitrogen in precipitate expressed as percentage of total nitrogen in sample
4.4	75.9
4.2	76.8
4.0	77.4
3.8	77.7
3.6	77.1
3.4	76.2

The greatest precipitation of protein was secured at pH 3.8. The remaining 22.3 per cent of the total horsebean nitrogen was divided between the extracted residue (5.8 per cent of the total nitrogen) and the supernatant solution (16.5 per cent of the total nitrogen). An amount of the nitrogen in the supernatant corresponding to 12.0 per cent of the total nitrogen of the sample was insoluble in 10 per cent trichloroacetic acid and was classified as non-protein nitrogen. This figure of 12.0 per cent for the non-protein nitrogen shows that the precipitate obtained by adjusting the alkali extract to pH 3.8 accounted for 88.3 per cent of the protein nitrogen present in the original material.

The optimal conditions for extraction and precipitation of a protein fraction as determined by the foregoing experiments were observed when preparing relatively carbohydrate-free protein precipitates for the cystine assays. Assays of acid hydrolysates of this protein fraction gave almost twice as high a figure for cystine in the whole bean as did assays conducted on direct acid hydrolysates of the meal.

METHODS USED IN MICROBIOLOGICAL ASSAYS

Methionine, leucine, isoleucine and cystine were assayed by Barton-Wright's (2) procedure using *Lactobacillus arabinosus* 17-5; lysine and histidine were assayed by the same procedure, but using *Leuconostoc mesenteroides* P-60. Arginine and threonine were assayed using Barton-Wright's (2) basal medium modified by including double amounts of glucose and sodium acetate; *L. arabinosus* 17-5 was used for the arginine assays and *Streptococcus faecalis* for the threonine assays. Proline, serine and aspartic acid were assayed by the procedure of Sauberlich and Baumann (11) using *L. mesenteroides* P-60. Tyrosine was assayed by the method of Baumgarten *et al.* (4) using *Streptococcus faecalis*; alamine was assayed by the same procedure, but with the modification that the glycine content of the basal medium was reduced from 200 mgm. per litre to 30 mgm. per litre in order to eliminate the initial flat section of the standard response curves. Phenylalanine and valine were assayed according to Horne *et al.* (8) using *L. arabinosus* 17-5, and glycine by the procedure of Dunn *et al.* (6) using *L. mesenteroides* P-60. Tryptophane was assayed by the method of Barton-Wright and Curtis (3), using *L. arabinosus* and extracting the hydrolysate thoroughly with ethyl ether and toluene prior to assay as a precaution against interference from indole and anthranilic acid.

TABLE 2.—AMINO ACID COMPOSITION OF HORSEBEAN (*Vicia faba* L.)

Amino acid	As per cent of whole bean containing 4.42% N	As per cent of horsebean crude protein containing 16% N
Alamine	0.73	2.64
Aspartic acid	1.79	6.46
Arginine	1.64	5.94
Cystine	0.30	1.09
Glutamic acid	3.78	13.68
Glycine	1.03	3.73
Histidine	0.80	2.90
Iso-leucine	1.53	5.54
Leucine	2.10	7.60
Lysine	1.53	5.54
Methionine	0.15	0.53
Phenylalanine	0.95	3.44
Proline	1.25	4.54
Serine	1.58	5.73
Threonine	0.73	2.64
Tryptophane	0.25	0.91
Tryosine	0.85	3.08
Valine	1.42	5.14

Experiments were conducted using increasing amounts of indole and of anthranilic acid instead of tryptophane in order to measure the responsiveness of *L. arabinosus* 17-5 to these analogues. The three substances gave almost identical response curves when supplied in equimolar amounts.

Glutamic acid assays were made using *L. arabinosus* 17-5 and the medium of Hac *et al.* (7) modified by including doubled amounts of glucose and sodium acetate, by using DL-aspartic acid at the rate of 67 mgm. per litre basal medium instead of 400 mgm. L-aspartic acid and by using L-glutamic acid to construct the standard curve.*

DISCUSSION OF ASSAY RESULTS

The complete assay results are summarized in Table 2.

Whole egg protein is known to be of excellent biological value for the chick. When the data for the horsebean are compared with the published data for whole egg proteins (5), as in Table 3, then it is evident that the methionine content of horsebean protein is relatively very low, and that its cystine content is also low. A better comparison from the standpoint of nutritional value for the chick is obtained when the amino acid percentages in the horsebean crude protein are compared with the amino acid percentages calculated for the crude protein of a theoretically adequate ration containing 20 per cent crude protein. The latter figures are based on Almquist's standards (1) for amino acid requirements of the chick, and the comparison is included in Table 3.

The data in Table 3 show that horsebean protein is likely to be very deficient in methionine and cystine if used as the sole source of protein in a chick ration. Glycine, phenylalanine and tryptophane fall below the optimal values given by Almquist (1), but the lysine content is adequate.

TABLE 3.—COMPARISON OF THE AMINO ACID COMPOSITION OF HORSEBEAN PROTEIN WITH THAT OF (*a*) THE PROTEIN OF WHOLE EGG AND (*b*) OF A PROTEIN THEORETICALLY ADEQUATE FOR THE CHICK AT A LEVEL OF 20 PER CENT CRUDE PROTEIN IN THE DIET

Amino acid	As per cent of horsebean protein containing 16% N	As per cent of whole egg protein containing 16% N	As per cent of theoretically ideal protein for chick nutrition (Almquist (1))
Arginine	6.5	6.4	6.0
Cystine	1.1	2.4	2.0
Glycine	3.7	2.5	5.0
Histidine	2.9	2.1	1.5
Isoleucine	5.5	6.0	3.0
Leucine	7.6	9.2	7.0
Lysine	5.5	7.2	4.5
Methionine	0.5	4.1	2.5
Phenylalanine	3.4	6.3	4.5
Threonine	2.6	4.9	3.0
Tryptophane	0.9	1.5	1.25
Tyrosine	3.1	4.5	—
Valine	5.1	7.3	4.0

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TABLE 4.—COMPOSITION OF BASAL RATION USED BY NICKOLAICZUK *et al.* (9)

Constituent	Per cent	Per cent of crude protein contributed by constituent, calculated from av. comp. (10)
Horsebean meal	35	52
Ground wheat	15	12
Hominy feed	20	11
Wheat middlings	10	9
Ground barley	10	7
Alfalfa meal	5	4
Brewers' yeast	2	5
Limestone	2	—
Iodized salt	1	—

Fortified fish oil	100 gm.	per 100 lb. ration
Riboflavin	0.08 gm.	
Manganous sulphate	6 gm.	

The inadequate proportions of phenylalanine and tryptophane do not mean that a marked growth response will follow the feeding of these amino acids along with horsebean protein, for the responses to any amino acid additions are likely to be obscured by the limiting effect of the low methionine content.

The most important aspect of this part of the discussion is the relation of the assay results to the feeling trial results secured by Nikolaiczuk *et al.* (9). These workers used a basal horsebean ration containing 33 per cent ground horsebeans. The composition of this basal horsebean ration is given in Table 4 since it is necessary also to consider the proportions of the total dietary protein supplied by the various ingredients.

Nikolaiczuk *et al.* (9) secured significant increases in rate of body weight gain when this ration was supplemented with DL-methionine, choline or DL-methionine plus choline, or when fish meal replaced part of the horsebean meal to an extent representing 2 per cent crude protein in the total ration.

Additions of tryptophane did not evoke any growth response, but additions of lysine evoked a response which approached statistical significance. In order more fully to interpret the results of Nikolaiczuk *et al.* (9) it is necessary to calculate the over-all amino acid composition of the basal horsebean ration and the basal horsebean ration plus fish meal, and to compare the data with Almquist's (1) standards for amino acid requirements. These calculations have been made using available data for amino acids as given by Block and Bolling (5) and the data for crude protein as given in the Quebec Feeders' Guide (10). The results are set out in Table 5.

The methionine content of horsebean seed and of the basal horsebean ration is very low. This low methionine content was clearly the limiting factor in determining growth on the basal ration. The addition of either methionine or of fish meal raised the methionine content of the ration to

TABLE 5.—COMPARISON OF AMINO ACID COMPOSITION OF HORSEBEAN SEED PROTEIN, OF THE PROTEIN OF THE BASAL HORSEBEAN RATION AND OF THE HORSEBEAN PLUS FISH MEAL RATION WITH THAT OF THE THEORETICALLY ADEQUATE CRUDE PROTEIN BASED ON ALMQUIST'S (1) STANDARDS

Crude protein of	Methionine	Lysine	Phenylalanine	Tryptophane
	%	%	%	%
Horsebean seed	0.5	5.5	3.4	0.9
Basal horsebean ration	1.2	4.0	4.1	1.1
Basal horsebean ration plus fish meal	2.2	4.3	4.3	1.2
Theoretically adequate ration containing 20 per cent crude protein (Almquist (1))	2.5	4.5	4.5	1.25

approximately the value of 2.5 per cent recommended by Almquist (1). This explains the remarkable increase in growth response which Nickolaiczuk *et al.* (9) obtained on adding 1.25 per cent methionine to their basal ration.

From Table 2 it can be seen that horsebean crude protein is adequate in lysine as compared with Almquist's "theoretical" protein. The crude protein of the basal horsebean ration, however, is slightly deficient for optimal growth (see Table 5). This suggests that additions of lysine to the basal horsebean ration would not be likely to give a growth response unless the ration was already supplemented with methionine. Nickolaiczuk *et al.* (9) obtained indications of a response to supplements of lysine, but the effect did not reach the level of statistical significance. The results of the present assays are, therefore, in keeping with the findings of Nickolaiczuk *et al.* (9).

Horsebean seed protein is somewhat deficient in phenylalanine. The basal horsebean ration, however, contains approximately 4.1 per cent phenylalanine against Almquist's (1) recommended optimum of 4.5. An addition of phenylalanine to the basal horsebean ration would have been unlikely to produce any dramatic increase in growth rate.

Horsebean seed protein itself has a rather low tryptophane content compared with Almquist's (1) standard requirement. The basal horsebean ration contains about 90 per cent of Almquist's (1) requirement figure, and the results, therefore, suggest that additions of tryptophane would be unlikely to produce a significant growth response unless the ration was already fortified with methionine. This inference is again in keeping with the experimental results secured by Nikolaiczuk *et al.* (9) where additions of tryptophane to the basal ration did not improve growth.

SUMMARY AND CONCLUSIONS

(a) The results of microbiological assays of the protein of the horsebean (*Vicia faba* L.) for eighteen amino acids are presented.

(b) A simple procedure is described whereby 88 per cent of the true protein of horsebean meal can be extracted with dilute alkali and reprecipitated relatively free from carbohydrate. Cystine assays on acid hydro-

lysates of this fraction gave results approximately half as great again as assays made on direct acid hydrolysates of the meal despite the loss of protein during isolation.

(c) The results are discussed in relation to existing data on the amino deficiencies of the horsebean when used as a source of protein in chick rations. Horsebean protein is relatively very deficient in methionine and cystine, and deficient to a lesser degree in tryptophane, glycine, phenylalanine and threonine. The analytical results confirm the finding of others that the nutritional deficiencies of horsebean meal as a source of protein in chick rations can be largely met by supplementing with methionine. Horsebean protein is likely to be adequate in arginine, histidine, isoleucine, leucine, lysine and valine if used as a sole source of protein for chicks.

ACKNOWLEDGMENTS

The authors wish to thank N. Nickolaiczuk, of the Department of Poultry, Macdonald College, for supplying the sample of horsebean, and for much helpful advice during the course of the work. They also wish to thank P. G. G. Gray and F. S. Thatcher, Macdonald College, for facilities and for advice on bacteriological matters.

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ERRATUM

In the paper "*Eradication of Poison Ivy.* IV." by W. H. Minshall, published in the December, 1949, issue, Vol. 29, No. 12, in Table 2 (page 587) the figure in the second line of the column headed "1942 Application" for "Sodium Chlorate" should read "50" instead of "0" as printed. This column should be as follows: "88, **50**, 47, 50".

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TESTING RESISTANCE TO SHATTERING AND LODGING IN CEREALS[1]

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INTRODUCTION

Over a period of years large annual losses are caused to the cereal crops of Western Canada by unfavourable weather conditions at harvest time. Wind, often accompanied by rain, causes shattering, stem breaking and lodging. The increased use of the combine for harvesting and the trend toward larger farm units result in crops often being left standing ripe for several weeks before they are harvested. Moreover, in an extremely favourable crop year, such as 1942, the abnormally large amount of grain to be harvested and the interference of wet weather with harvesting operations may result in tens of thousands of acres of crop being exposed uncut for long periods after the grain is ripe.

Many factors concern the ability of varieties to hold their grain in a recoverable position for a period of several weeks after maturity. While the problem is complex, shattering and lodging are so important commercially that any real improvement in existing methods of appraising varieties and selecting hybrids for shattering and lodging resistance should be of considerable value to both farmers and cereal breeders. Accordingly, a study was commenced in 1946 at the University of Saskatchewan with the financial assistance of the National Research Council. The present paper is a progress report on the first three years of work.

LITERATURE REVIEW

The importance of root development in the resistance of plants to lodging has been emphasized by numerous investigators. Derick and Hamilton (4) and Caffery and Carroll (2) working with oats, and McRostie and MacLachlan (8), Hayes and McClelland (6) and Hall (5) working with corn, found strong root development with substantial anchorage was closely related to lodging resistance.

Many investigators have studied the culm of cereals to learn more about varietal resistance to lodging. Welton and Morris (10) considered carbohydrate content at heading time an important factor in determining the habit of growth and consequently a leading factor involved in lodging; Willis (11) found breaking strength of the straw related to lodging, and Atkins (1) found unit weight of straw sections to be as reliable an index as breaking strength.

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Various studies have been made on methods of testing shattering in cereals. Jung (7) estimated shattering from heads dropped on a glass plate. Vogel (9) and Chang (3) found that tightly attached glumes allowed less shattering to occur. Chang (3) also classified varieties for shattering after beating the heads in a special machine.

MATERIALS AND METHODS

Varieties ranking from high to low for resistance to lodging and shattering were used in each crop with a total of 21 of wheat, 18 of barley and 10 of oats. Replicated sowings in different sized microplots at different dates and under different conditions, including irrigation, were made each year at Saskatoon. Various methods were devised and machines constructed to test single plants as well as entire plots for shattering and lodging. Of the 14 methods tested, 11 proved unsatisfactory and three showed distinct promise.

METHODS PROVING UNSATISFACTORY

1. *The wind machine* is a device for exposing field microplots individually to a strong controlled wind caused by an aeroplane propeller driven by an automobile engine. This machine was used extensively for three years with various wind velocities and exposure periods. The results showed the device to have some value but on the whole to be too slow, cumbersome and limited by weather conditions to be worth-while.

2. *Hygroscopicity* of the chaff and grain were studied through the use of Petri dishes, dessicators, a large incubator and quantitative scales. These tests were comprehensive, yet gave no significant differences between varieties.

3. *Spikes rubbing together*, as in a wind, was achieved with a device in which the heads, fixed in upright positions, were caused to rub together. Although tried with many modifications, this machine proved quite unsatisfactory.

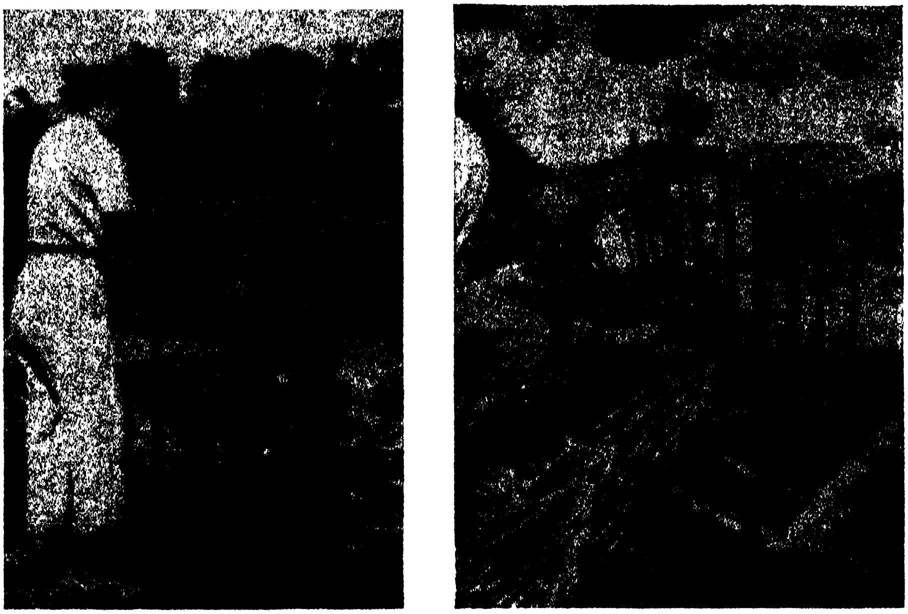
4. *Compressed air* was used to secure a vigorous agitation of heads in a metal cylinder, the air being directed into the cylinder by a nozzle. Various air pressures were used but the results did not agree sufficiently well with field shattering to be considered reliable.

5. *A corrugated wire drum*, in which heads of grain were tumbled about by hand crank rotation of the drum, gave results of some value. However, the work was very time-consuming and the device was considered impractical.

6. *A corrugated metal drum* lined with metal cleats and rotated by a hand crank gave results which agree fairly well with field performance; but the method was considered too time-consuming to be of practical value.

7. *Alternate wetting and drying* of heads in the nursery plots using a portable sprayer gave results of no value.

8. *Nutrient solution* tests in which several varieties of each crop were grown in gallon jars of nutrient solution in the greenhouse for comparison of root and crown development proved disappointing. The results suggested that a fibrous, much-branched root system was associated with lodging resistance, but the data were inconclusive statistically.



FIGURES 1 and 2. Root washing, preliminary and final stages.

9. *Lodging induced* by pressing each row of plants down with a board and then letting the material recover as best it could showed no correlation with the known lodging behaviour of the varieties tested.

10. *Culm weight.* Atkins' (1) method of weighing a hundred comparable stem portions of different varieties and correlating the weights with field lodging results was tested thoroughly. The tests were continued for three years but the results proved of little real value.

11. *Root pulling resistance* was obtained by tying a cord to a plant at its base and pulling it out vertically with a recording spring balance. In 1946, the first year of this investigation, significant varietal differences were obtained and the method seemed promising. However, in the two succeeding years, although the sampling and testing techniques were made as efficient as possible, the results were disappointing. The method was deemed unsatisfactory as a means of testing tendency to lodge.

METHODS SHOWING PROMISE

1. *Glume pulling.* Strength of glume attachment was tested by Vogel's (9) method, which consists of using a small spring balance to loosen a glume. A preliminary study, in which ten heads of each of seven varieties were used, showed a general agreement between grams pulling resistance and the known shattering resistance of the varieties. A progressive increase in strength of glume attachment from the second to the basal spikelet was also apparent.

The method was then applied to all of the varieties of wheat with the results given in Table 1. The data show that in all varieties there was a general increase in glume strength toward the base of the spike. The strength of attachment of the glumes was greater in the durum wheat

TABLE 1.—THE NUMBER OF GRAMS PULL REQUIRED TO REMOVE THE GLUMES FROM EIGHTEEN VARIETIES OF WHEAT*

Varieties	Spikelet number from top to bottom of spike												Mean results for spikelets			
	1	2	3	4	5	6	7	8	9	10	11	12	2 and 3	9 and 10	2, 3, 9 and 10	All
Pelissier	23.4	24.2	28.2	32.3	36.2	41.2	41.5	51.5	51.1	60.8	64.9	63.0	26.2	55.9	41.0	43.2
Mindum	15.3	11.4	12.8	13.4	18.0	19.4	21.4	23.1	24.8	27.4	28.0	35.6	12.1	26.1	19.1	20.9
Stewart	22.0	15.2	22.2	25.0	26.0	28.0	32.8	37.5	39.1	41.2	45.0	53.6	18.7	40.1	29.4	32.3
Thatcher	29.8	7.7	8.5	10.2	12.8	12.6	19.0	23.3	28.1	31.7	33.7	38.5	8.1	29.9	19.0	21.3
Marquis	26.4	1.5	2.8	7.6	13.0	12.6	20.2	23.6	26.8	32.8	36.2	43.0	2.1	29.8	15.9	20.5
Cadet	32.5	6.6	8.1	9.5	16.0	16.5	21.4	25.5	27.9	25.6	26.2	25.6	7.3	26.7	17.0	20.1
Apex	24.5	1.4	3.2	7.0	8.8	13.4	16.8	23.8	26.8	28.2	32.4	33.2	2.3	28.0	15.1	18.3
Renown	22.4	1.6	4.0	6.0	11.5	15.4	23.4	29.0	36.2	38.9	42.2	47.2	2.8	37.5	20.6	23.2
Reward	15.8	1.0	1.2	3.2	5.5	8.7	13.0	15.2	23.4	27.0	29.9	33.6	1.1	25.2	13.1	14.8
Regent	14.4	1.4	2.3	3.5	5.1	9.1	11.4	14.6	18.8	20.4	21.7	21.2	1.8	19.6	10.7	12.0
Rescue	19.4	2.9	4.8	4.9	7.0	8.2	9.9	9.5	10.9	10.8	11.9	13.6	3.8	10.8	7.3	9.5
Comet	53.4	9.2	15.4	21.5	24.2	28.0	35.0	36.2	39.0	40.9	50.2	65.1	12.3	39.9	26.1	34.8
Reliance	35.4	12.0	17.3	18.8	19.1	23.2	25.4	29.6	32.7	36.1	33.4	41.6	14.6	34.4	24.5	27.1
Red Bobs	8.0	1.0	1.2	1.2	1.7	1.6	3.2	3.6	5.6	6.2	6.6	8.0	1.1	5.9	3.5	4.0
Garnet	16.0	1.0	1.3	2.6	4.5	5.0	7.5	8.3	12.8	13.6	14.4	12.4	1.1	13.2	7.1	8.3
Huron	12.6	1.9	3.2	5.4	5.7	7.6	8.6	9.8	11.0	11.9	12.7	14.7	2.5	11.4	6.9	8.8
Pioneer	15.6	1.0	1.3	1.6	1.6	3.5	4.6	5.0	5.9	7.2	7.6	8.1	1.1	6.5	4.3	5.2
Mercury	35.8	4.4	6.6	8.8	9.8	14.4	19.2	22.4	22.5	26.6	23.0	21.0	5.5	24.5	15.0	17.9

* Each figure is the average for the first and second glume of the designated spikelet of each of ten heads.

varieties than in the common wheat varieties. In general the pulling resistance of the glumes agreed with the known shattering resistance of the varieties. However, there was some lack of agreement. For example, Thatcher, which resists shattering far more than Mercury, was similar to that variety in glume pulling resistance.

A study of the varietal differences for different parts of the head revealed that the average for the second and third spikelets from the top of the head together with the average for the third and fourth spikelets from the bottom, gave about as good a picture of varietal performance as the results from the entire heads. These averages are shown with the general averages in the last four columns of Table 1.

2. *The paddle device.* In 1948, a head-striking machine essentially similar to that described by Chang (3) was constructed. With this device ripe heads are subjected to the striking or beating action of a plywood paddle, actuated by a motor-driven eccentric. The weight of grain threshed out of thirty heads treated in groups of three was obtained for each of 18 varieties of wheat. The data, converted to percentage shattered, are presented in Table 2.

The shattering results of the paddle method did not agree exactly with the known behaviour of the varieties but the grouping of the resistant as compared with the susceptible varieties was good. This method is quite promising.

TABLE 2.—COMPARATIVE SHATTERING RESULTS ON WHEAT VARIETIES AS OBTAINED WITH VARIOUS LABORATORY AND FIELD DEVICES AND IN THE REPLICATED NURSERY PLOT TESTS

Variety	Summarized* SRC**	Field shattering 1948, Saskatoon		Strength† of attachment of glumes		Shattering induced by paddle device	
		Per cent	SRC	Grm.	SRC	Per cent	SRC
Pelissier	VH	0.5	H	43.2	VH	0.0	VH
Stewart	VH	1.0	H	32.3	VH	1.9	H
Mindum	H	6.0	M	20.9	H	8.6	MH
Thatcher	H	1.0	H	21.3	H	3.3	H
Saunders	H	0.5	H	—	—	4.7	H
Redman	H	1.5	H	—	—	5.7	H
Marquis	H	2.0	H	20.5	H	10.6	MH
Cadet	H	2.0	H	20.1	H	13.1	MH
Apex	H	2.0	H	18.3	H	7.3	MH
Renown	H	—	—	23.2	H	—	—
Reward	H	—	—	14.8	MH	—	—
Regent	H	3.0	MH	12.0	MH	3.0	H
Rescue	H	4.0	MH	9.5	MH	9.3	MH
Comet	H	—	—	34.8	VH	—	—
Reliance	M	7.0	M	27.1	H	6.5	MH
Red Bobs	ML	2.0	H	4.0	L	10.3	MH
Garnet	ML	16.0	L	8.3	L	15.2	ML
Huron	L	25.0	L	8.8	L	22.2	ML
Pioneer	L	31.5	L	5.2	L	23.0	ML
Prelude	L	23.0	L	—	—	17.2	ML
Mercury	L	15.0	L	17.9	MH	57.0	L

* Using all available data for several years.

** SRC = Shattering Resistance Class.

† Average of primary and secondary glumes of each of the 2nd, 3rd, 9th and 10th spikelets from the top of each of ten heads.

Abbreviations: VH—Very high; H—High; MH—Medium high;
M—Medium; ML—Medium low; L—Low.

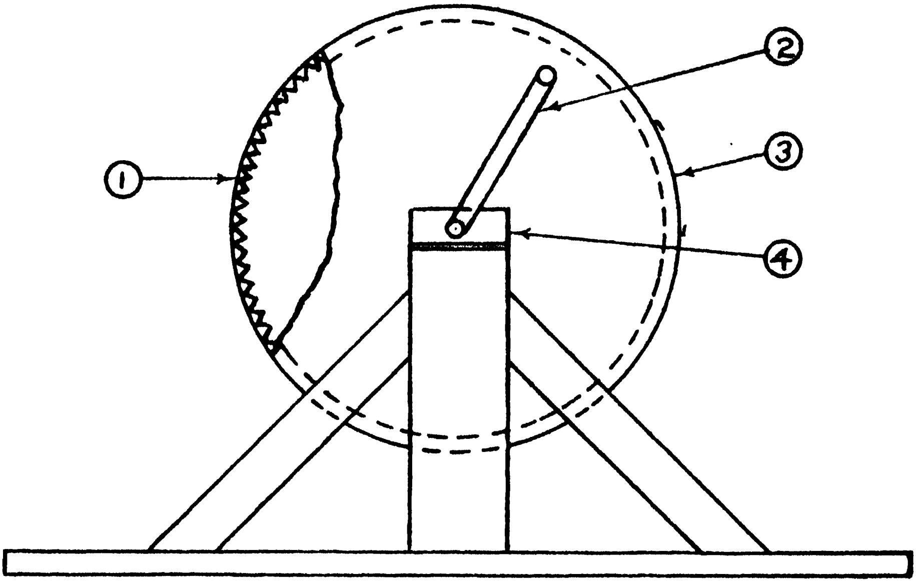
3. *Root washing method.* As a follow-up to the nutrient solution root studies of 1947, an experiment was devised to determine whether any relationship existed between root placement and lodging. Varieties representing a wide range of resistance to lodging were used, the seeds being sown 12 inches apart each way at a uniform depth. Ten plants in each of the following varieties were examined: Glacier, Newal, Vantage, Trebi, and Byng barley; Thatcher, Apex 1789, and Pioneer *vulgare* wheat; Carleton *durum* wheat and Prolific spring rye. The procedure used was to excavate a plant and its roots, taking up a block of soil one foot square and six inches deep. The block was placed in a wooden box and allowed to soak in water for several hours. A number of small, stiff rods were driven through each block from the side to prevent the roots from moving in one plane. The wooden box was then removed and the block was washed out with a fine spray. When washing was completed, the roots were flat upon a wooden plate. A comparative study of the roots showed no definite relation between root placement and the lodging resistance of the varieties concerned. In addition there appeared to be no correlation between the possession of a fibrous, much branched root system and resistance to lodging, although the preliminary greenhouse study suggested such a relationship.

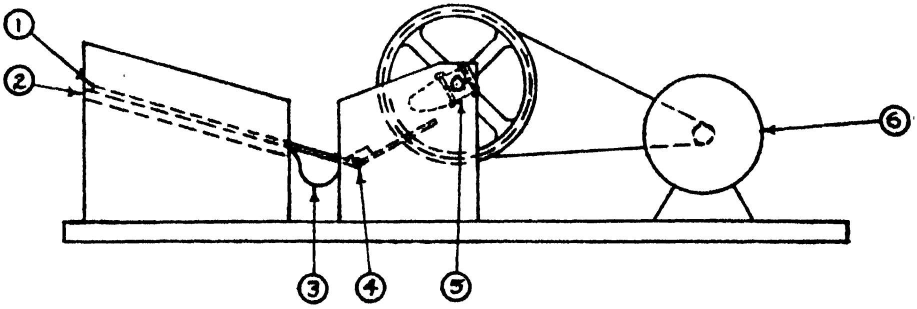
The second portion of the field experiment involved taking measurements of the following characters of the plants: height of the tallest culm; the average height of all culms; average culm diameter taken one inch above the second above ground internode; number of coronal roots; circumference of the crown; dry weight of top growth; dry weight of root growth. Indices were made of the ratio of the weight of top growth to the weight of root growth and of the ratio of crown circumference to the number of culms. The data for the ten plants of each variety averaged for each character are given in Table 3.

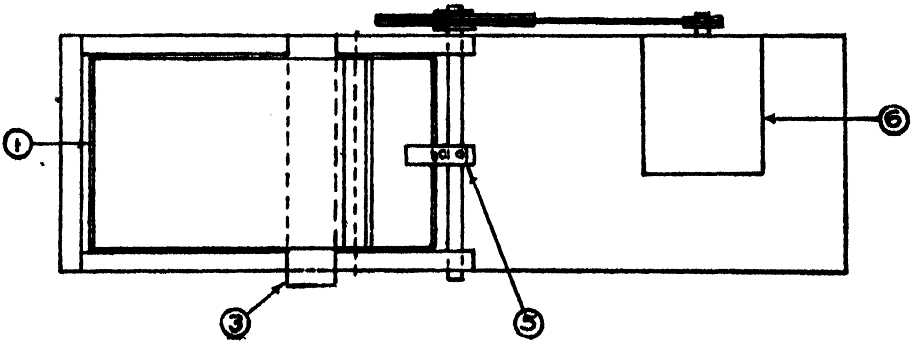
The results indicated a relationship between the number of coronal roots and lodging resistance, that is, the greater the number of coronal roots the greater the resistance to lodging. None of the other characters appeared to be correlated with lodging resistance. The variance analysis of the data on number of coronal roots showed highly significant varietal differences in wheat with Thatcher having more coronal roots than Apex and Apex more than Pioneer. Among the barley varieties there was a highly significant difference between the number of coronal roots of Glacier and of the two varieties Trebi and Byng and a significant difference (at the 5 per cent level) between Glacier and the varieties Vantage and Newal.

DISCUSSION

Many environmental factors concern the ability of a variety to hold its grain in a recoverable position for a period of several weeks after maturity. This makes it extremely difficult to devise simple, quick tests which aid the breeder in differentiating varieties or hybrids as to this ability. The method used to obtain estimates of shattering or of lodging resistance must be suitable for different seasons and locations. Several of the various techniques and devices tested during the course of these investigations have proven of some value and at least three of them have distinct promise. However, most of them have been found to be too laborious or too unreliable for practical use.







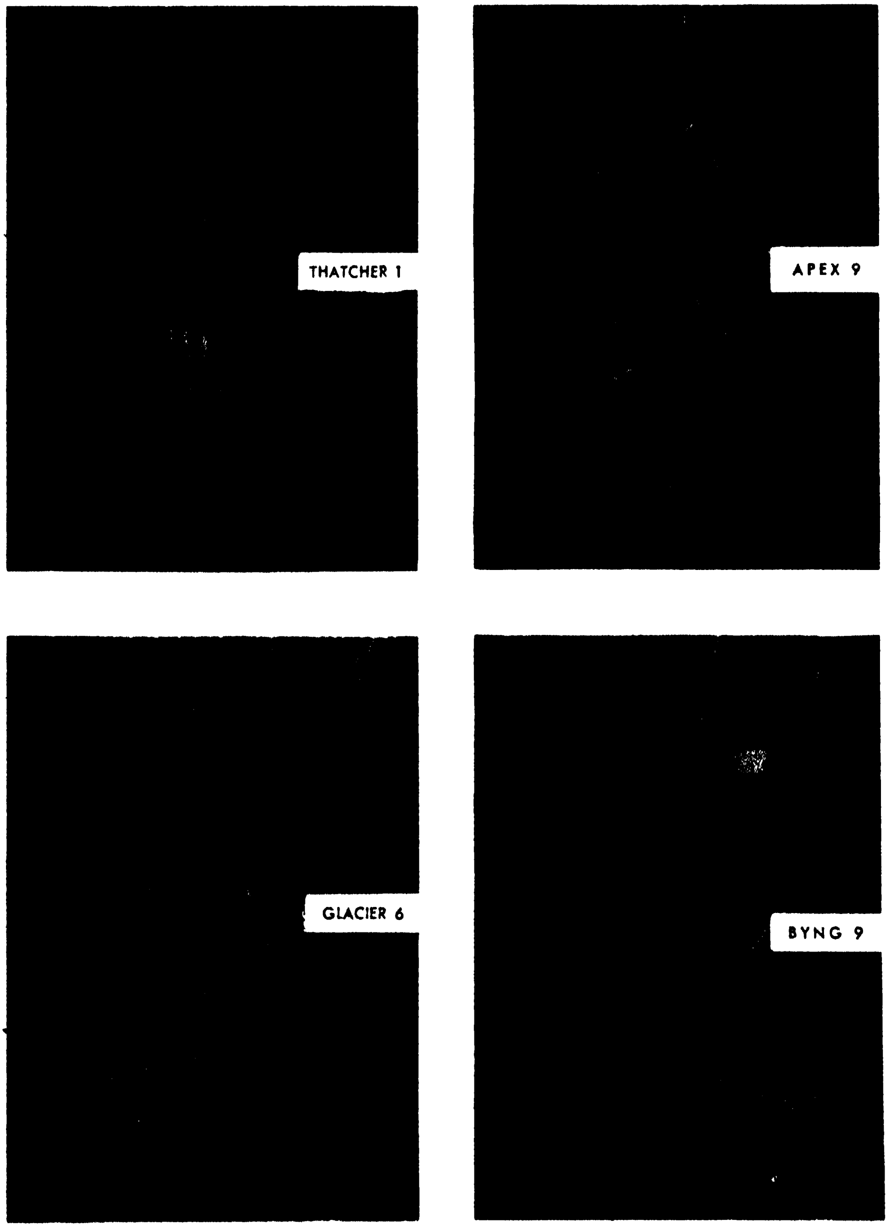
FIGURES 3, 4 and 5.
Top (Figure 3)—Corrugated metal drum. (1) corrugations; (2) crank handle; (3) door, and (4) angle iron support and bearing.
Centre (Figure 4)—Paddle device (legend as below).
Bottom (Figure 5)—Paddle device. (1) plywood paddle; (2) table; (3) metal trough; (4) pivot; (5) eccentric, and (6) electric motor.

TABLE 3.—SUMMARY OF DATA FROM CEREAL ROOT STUDIES

Variety*	Average height of tallest culm (cm.)	Average height of all culms (cm.)	Average number of culms	Average culm diam. (mm.)	Average number of coronal roots	Average weight of top growth (gm.)	Average weight of root growth (gm.)	Ratio top growth	Average crown circum. (mm.)	Ratio crown circum.
								Root portion†		Average number of culms
Wheat										
Thatcher	72.75	67.0	16.9	2.82	73.2	19.5	0.54	36.1	55.5	3.28
Apex	78.05	69.4	17.0	2.91	61.0	22.3	0.67	33.3	56.3	3.31
Pioneer	72.80	67.5	11.8	3.02	51.2	14.9	0.30	50.0	47.9	4.06
Barley										
Glacier	70.90	66.7	15.1	3.78	67.0	23.3	1.82	12.8	61.8	4.09
Vantage	63.35	57.6	10.7	3.79	58.2	20.8	1.27	16.4	56.5	5.28
Newal	75.70	65.8	18.4	3.70	57.3	37.3	2.52	14.8	64.5	3.51
Trebi	60.50	55.7	14.3	3.47	54.5	22.5	0.82	27.4	59.4	4.15
Byng	76.90	66.3	20 1	3.72	54.5	32.8	2.37	13.8	63.2	3.14

† Weight ratio total top growth to portion of roots in soil 12 inches × 12 inches × 6 inches deep at plant's base.

* The varieties are arranged in order from the most to the least resistant to lodging according to nursery results, 1942 to 1948.



FIGURES 6 to 9. Representative plants of Thatcher and Apex wheat, and Glacier and Byng barley.

The results obtained on shattering indicate that this character is complex and is influenced by various factors and combinations of circumstances. The method of recording shattering, as carried on at Saskatoon for a number of years*, by taking notes on border rows or special plots left standing for several weeks after harvest, appears to be more practical than any of the methods proposed in the literature. Nevertheless, results with the paddle device and the pulling resistance of wheat glumes proved to be fairly closely related to the known ability of varieties to resist shattering. The results suggest that either the paddle device or the glume pulling test could be used with a large degree of success in separating varieties as to shattering propensity under conditions which make inconvenient or impossible the taking of after-harvest shattering notes in the nursery.

The studies on lodging reveal that this character is also complex and much affected by environmental influences. Of the different methods used to evaluate varieties for lodging resistance, apart from taking field notes under propitious circumstances, such as in an irrigated nursery, the coronal root counting procedure proved to be the most promising. This method, while involving digging up and washing out the base of the plant and the adjacent roots, seems to have real merit.

A study of the promising procedures brought to light in this research project indicates that each of them can be simplified for application to hybrid lines. By using fewer heads per sample in the paddle device and by estimating rather than weighing the seed dislodged from the heads, the procedure could be used economically on hundreds of head samples. This device, with some adjustment, appears suitable to use on barley as well as on wheat. The data on glume pulling resistance show that satisfactory representation of a variety glume adherence may be obtained from the pulling resistance of the glumes on the second and third spikelets from the top of the head together with that of the third and fourth spikelets from the bottom of the head, and that an eight or ten head sample per variety is sufficient. This abbreviated procedure is sufficiently quick and economical to be used for testing hybrid lines. Finally, the elaborate, painstaking method developed for ascertaining the placement and number of coronal roots can be simplified by taking a smaller soil block, washing it without incasing it or using the rods and omitting all measurements excepting the count of coronal roots. These changes would allow the method to be used on hybrid lines.

The investigations of the past three years as herein reported are to be followed up by detailed exploitation of the merits of each of the three promising methods.

SUMMARY

1. Fourteen methods and devices were used in an endeavour to develop better procedures for determining the relative resistance of varieties of cereal crops to shattering and lodging.

2. Three of the methods showed promising results. These are: (a) determining glume pulling resistance to obtain an estimate of shattering resistance; (b) beating grain heads with a paddle to determine shattering resistance directly, and (c) counting the number of crown roots to obtain an indication of lodging resistance.

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* Developed by the Field Husbandry Department, University of Saskatchewan, during the period 1940 to 1945.

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THE DETERMINATION OF PHOSPHORUS IN SODIUM ACETATE EXTRACTS OF SOIL[1]

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INTRODUCTION

The use of a sodium acetate solution as extractant in a system of rapid tests for available plant food constituents in soils was first introduced by Morgan (2) in 1935 under the designation of the "Universal soil extracting solution". Since that time, this solution has been used in soil fertility investigations by many workers in various parts of the world. In 1944, in a study of rapid microchemical soil tests, Peech and English (3) indicated that the "solution proposed by Morgan has certain desirable features not possessed either by solutions of strong acids or by solutions of neutral salts" and they presented details of improved chemical methods for the determination of constituents extracted from soil by Morgan's solution.

PREPARATION OF EXTRACTING SOLUTION

In the determination of phosphorus in such extracts, the molybdenum blue colours are usually compared visually with those of a set of standards prepared at the same time. In this laboratory, it was desired to make the readings in a Klett-Summerson photoelectric colorimeter. While such a procedure may be slightly more time-consuming, it is more accurate and obviates the necessity of running standards with every series of determinations. It was observed, however, that the blank reading obtained when only the reagents were used was quite large and this lessened the accuracy of the determination, particularly with soils of low phosphorus content. Attempts were made to reduce the value of the blank reading in two ways: (1) by purification of the activated carbon used to remove soluble organic matter, by boiling in hydrochloric acid, keeping warm on the hot plate overnight and washing repeatedly by decantation with large volumes of distilled water; (2) by making the extracting solution from sodium hydroxide and glacial acetic acid rather than from the sodium acetate salt. That these treatments were successful in reducing the magnitude of the colorimeter reading for the blank is shown by the following results, where the molybdate reagent used was made up in hydrochloric acid and stannous oxalate was used as the reducing agent:

Reading for solution made from $NaC_2H_3O_2 . 3H_2O$, using unpurified carbon—39;

Reading for solution made from $NaC_2H_3O_2 . 3H_2O$, using purified carbon—27;

Reading for solution made from $NaOH + HC_2H_3O_2$, using purified carbon—12.

It has been found that the value of the blank does vary between different lots of sodium acetate. Greater accuracy in the determinations can be expected when a low blank reading is obtained.

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INTERFERENCE BY ARSENIC

One difficulty in all phosphorus determinations which depend on the development of the molybdenum blue colour is due to the fact that arsenic, when present in the arsenate form, gives the same colour. In many soils, particularly those from areas where arsenate spray residues have accumulated, this leads to somewhat high values for phosphorus. However, it has been shown (4) that arsenic in the arsenite form does not give a blue colour and this has suggested a means of eliminating the arsenate interference by reduction before the colour is developed. This reduction can be done by means of sodium bisulphite and this has been used by several workers (5, 6) under varying conditions.

In a proposed method for the determination of the total phosphorus content of a soil in which extraction is made with 60 per cent perchloric acid, Sherman (5) stated that the development of the blue colour is affected by acidity, temperature and time, and recommended a minimum of 2.5 ml. of perchloric acid in a 25 ml. volume and a temperature of 25° ± 4° C., with readings being taken between 15 and 25 minutes after the reagents were mixed. When arsenic was present, solid sodium bisulphite was added and the solution allowed to stand for three hours before the colour was developed, using aminonaphtholsulphonic acid as reducing agent.

In this laboratory, the Sherman procedure has been modified so that it can be used in measuring the phosphorus extracted from a soil by Morgan's solution. To a suitable aliquot of the extract, 2.5 ml. of perchloric acid is added, followed by dilution with water and cooling. Sodium bisulphite is then added in solution and the mixture allowed to stand overnight. In the morning, the aminonaphtholsulphonic acid and ammonium molybdate are added and the colour read after 15 minutes.

When the sodium bisulphite was added in the solid form, a turbidity was obtained, necessitating centrifuging before the final development of the colour. When this compound was added in solution, no such turbidity was obtained. The making of a solution requires only one weighing and the measurement of a definite volume for each determination is relatively simple and rapid.

The importance of having the reaction of the solution in which the colour is developed sufficiently acid has been stressed by Holmes and Motzok (1) who have indicated that measuring acidity in terms of pH value rather than normality is preferable. It was shown that, when aminonaphtholsulphonic acid was used as the reducing agent, the range of acidity should be between pH 0.05 and pH 0.60, and this finding has been substantiated by work in this laboratory. In the procedure outlined below, the pH value of the final solution is approximately 0.35.

DETAILS OF PROPOSED METHOD

The details of the modified method are as follows:

Reagents

Extracting solution. Dissolve 58.54 gm. of NaOH (C. P. grade) in water. Cool. Add 145.8 ml. of 99.5 per cent acetic acid. Make to 2 litres. If necessary, adjust to pH 4.85.

Activated carbon. Darco G 60, purified by boiling with 6 N HCl, keeping warm on the hot plate overnight, and washing repeatedly by decantation with large volumes of distilled water. The carbon is recovered from suspension by filtering on a Buckner funnel. It is then dried at 118° C. overnight.

Perchloric acid. Seventy-two per cent, phosphorus-free.

Sodium hydrogen sulphite solution (15 per cent)—Dissolve 15 gm. $NaHSO_3$ in distilled water, filter and make to 100 ml.

1,2,4-*Aminonaphtholsulphonic acid.* Add 0.25 gm. of this material to 88 ml. of 15 per cent $NaHSO_3$ solution. Then add, slowly and with shaking, a 20 per cent solution of sodium sulphite (Na_2SO_3) until the solution is clear. (This usually takes from 5 to 7 ml. Up to 4 ml. can be added in 1 ml. portions, the remainder drop-wise.) Filter and store in a dark bottle. Prepare fresh every week.

Ammonium molybdate. Dissolve 5 gm. $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 100 ml. distilled water. Let stand overnight. Filter if necessary.

Standard phosphate solution. Dissolve 0.4390 gm. KH_2PO_4 in 1 litre of extracting solution. This solution contains 100 p.p.m. P. Dilute 100 ml. of this to 1 litre to make a solution containing 10 p.p.m. P.

Procedure

To 10 gm. of soil (air-dried and screened through a 2 mm. sieve) in a shaking bottle, add ¼ measuring teaspoonful (¼ gm.) of purified activated carbon and 50 ml. of the extracting solution. Shake for 30 minutes and filter into a 50 ml. Erlenmeyer flask through a dry 15 cm. Whatman No. 42 filter paper.

Transfer 10 ml. of filtered extract (representing 2 gm. of soil) to a 50 ml. glass-stoppered graduated cylinder. Add 2.5 ml. of perchloric acid and mix. Dilute with distilled water to a volume of 18 ml., mix and let cool. Add 2.5 ml. of 15 per cent $NaHSO_3$ solution, mix thoroughly, stopper and let stand overnight.

Add 0.8 ml. of 1,2,4-aminonaphtholsulphonic acid solution and mix. Add 2 ml. of ammonium molybdate solution, and water to a volume of 25 ml. Mix thoroughly. Allow 15 minutes for the development of the colour and read in a photoelectric colorimeter, using a red filter.

Calculate the amount of phosphorus from a curve prepared as follows: Measure aliquots (100.0, 80.0, 60.0, 40.0, 20.0, 10.0, 5.0, 0.0 ml.) of the standard (10 p.p.m. P) phosphate solution into a series of 100 ml. volumetric flasks. Dilute to the mark with extracting solution and mix thoroughly. Pipette 10 ml. of each into 50 ml. glass-stoppered graduated cylinders and continue as in the second and third paragraphs under "procedure".

ELIMINATION OF ARSENIC INTERFERENCE

The effect of arsenic on the apparent soluble phosphorus content of a soil, and the elimination of this effect by the procedure outlined above, is shown by the following experiment. Two soils well supplied with phosphorus soluble in the Universal extracting solution were treated in the laboratory with increasing amounts of "insecto" containing 6.5 per cent As as calcium arsenate ($Ca_3(AsO_4)_2$). After moistening and drying, the samples were extracted in the usual manner and the phosphorus in the extract determined both with and without treatment with sodium bisulphite to eliminate the effect of the arsenic. The results obtained are presented in Table 1.

It is readily seen that, with both soils, increasing amounts of arsenic have resulted in increased values for the apparent phosphorus content when no sodium bisulphite was used. However, when the arsenic was reduced, the values for all five samples of each soil were approximately the same, indicating that the effect of the arsenic had been eliminated. In the the case of soil B, these values were about the same as that for the sample to which no arsenic had been added and where the colour was developed without the use of $NaHSO_3$. With soil A, however, the values obtained when $NaHSO_3$, was used were somewhat lower than that for the sample receiving no added arsenic and where no $NaHSO_3$ was used. This suggests the presence of arsenic in the original sample of soil A and this may well have been the case, since the sample was taken from an area on which potatoes have been grown frequently during the past 15 years and where arsenate spray residues may have accumulated.

SUMMARY

In the colorimetric determination of phosphorus in soil extracts, care should be taken to eliminate interference by arsenic which gives a blue colour similar to that due to phosphorus. This can be done by reducing the arsenate to the arsenite form before development of the colour. A procedure for determining phosphorus in the presence of arsenic has been adapted for use with the sodium acetate (Universal) soil extracting solution. Data are presented for soil samples to which arsenic has been added, showing that the proposed method is satisfactory.

TABLE 1.—DETERMINATION OF PHOSPHORUS IN EXTRACTS OF SOILS TO WHICH ARSENIC WAS ADDED

Soil	Arsenic added (p.p.m.As)	Apparent phosphorus content (lb. P per ac.)	
		Without $NaHSO_3$ treatment	With $NaHSO_3$ treatment
A	0	34	25
	10	37	25
	20	41	25
	40	54	26
	60	68	28
B	0	33	30
	40	41	32
	60	52	32
	80	56	33
	100	76	35

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THE USE OF MONOSOMES AND NULLISOMES IN CYTOGENETIC STUDIES OF COMMON WHEAT[1]

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INTRODUCTION

Viable chromosomal aberrations involving the loss of a portion of a chromosome, an entire chromosome, or even a chromosome pair provide a method of associating genes with certain chromosomes in some and possibly most polyploid species. This report deals with the use of 17 different nullisomic lines of common wheat in studying the genetics of glume colour, awning, spike density, habit of growth, leaf rust reaction and bunt reaction.

REVIEW OF LITERATURE

Cytologic and Cytogenetic Studies of Deficiencies for Whole Chromosomes, Pairs of Chromosomes, and Chromosome Arms

Cytologic Studies of Monosomics

Monosomics are plants deficient for an entire chromosome, and they have been obtained in several polyploid species. In *Nicotiana tabacum*, Clausen (15) reported obtaining 20 of the possible 24 monosomics and demonstrated their usefulness in associating genes with whole chromosomes. Transmission of whole chromosome deficiencies was studied by Olmo (41) in *N. tabacum* and by Lammerts (30) in *N. rustica*. In both species the deficiency was found to be transmitted with a high frequency through the eggs but only rarely through the pollen.

Whole chromosome deficiencies in common wheat have been reported by numerous investigators. However, the chromosome involved usually was associated with speltoidy of the B type (26, 36, 71). Love obtained several different monosomics in progenies of crosses between *T. durum* and *T. vulgare*. Kihara (29) found monosomics in derivatives of *T. polonicum* × *T. spelta*, and described the behaviour of univalent chromosomes at meiosis. Nishiyama (40) studied transmission of the deficiency in crosses between 2 different nullisomics with *T. spelta* and found 73 per cent effective female and 11 per cent effective male gametes to be n-1. Powers (45), and Myers and Powers (37) reported that meiotic instability and genetic irregularities often were associated with the monosomic condition.

Cytologic Studies of Nullisomics

Nullisomics are plants deficient for a pair of chromosomes. None was obtained by Clausen (15) in *N. tabacum*. Lammerts (30) suggested that viability of n-1 gametes and 2n-2 zygotes is evidence that a species is highly polyploid.

Nullisomics, obtained in the progeny of heterozygous speltoids, have been described in detail elsewhere (26, 36, 71). A few nullisomes, other than those resulting in speltoidy, also have been found (29, 33).

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It remained for Sears, however, to obtain a considerable number of different nullisomics in one variety. In his first of a series of papers (50), the origin of the deficiencies was related. They were obtained by pollinating a haploid plant of Chinese Spring with normal pollen. He postulated 4 ways in which the deficiencies could have arisen, and suggested that these deficiencies could be used for associating with specific chromosomes some of the genes of common wheat. Later (51), he reported briefly on 7 nullisomics obtained up to that time. All exhibited reduced vigour and fertility, but all were female fertile, and 4 produced functional pollen. Nullisomic line III, which was partially asynaptic, was suggested as a probable source of additional deficiencies. In 1944 he gave a comprehensive report (52) on 17 different nullisomics then available. He numbered the nullisomics I to XI in the order in which they were obtained if the nullisome corresponded to a particular chromosome of the Emmer (tetraploid) wheat series. Those that corresponded to a particular chromosome of the C genom of the common (hexaploid) wheats were numbered XV and up in the order in which they were obtained. He found that nullisomics represented from 0.9 to 10.3 per cent and monosomics 75 to 85 per cent of the progeny of the different monosomics.

Cytologic Studies of Plants with Telocentric Chromosomes and Isochromosomes

Telocentric chromosomes are deficient for an entire arm and have a terminal centromere. Isochromosomes are deficient for one arm and reduplicated for the other. Darlington (17) has shown that telocentric chromosomes were formed by misdivision of the centromere, and isochromosomes from the reduplication of such broken chromosomes.

The usefulness of a telocentric chromosome has been demonstrated in genetic studies of maize by Rhoades (47). Smith (56) indicated how they could be used in linkage studies of *T. monococcum*. Love (34) showed that telocentric chromosomes resulted in two different off-type "mutants" in the variety Dawson's Golden Chaff. In the case one mutant, the arm lost normally carried the gene for red chaff in that variety, since plants homozygous for the deficiency had white chaff. He believed these aberrations to be quite stable in common wheat, contrasting with the situation in maize (47). The function of telocentric chromosomes and isochromosomes, in changing plants from normalcy through speltoidy to compactoidy at various dosage levels, has been discussed by Sanchez-Monge and MacKey (49), and Li *et al.* (31). Sears (52, 53) found that isochromosomes and telocentric chromosomes frequently occurred in the progeny of certain monosomics. In somatic tissue, telocentric chromosomes were found to be less stable than isochromosomes. The latter had approximately the same frequency of loss and misdivision as primary monosomes.

Cytogenetic Studies with Chromosome Deficiencies

Sears (52) used monosomes to determine the location of certain genes. He found that a gene for red seed in Chinese Spring was carried by chromosome XVI. The B-type of speltoidy was associated with chromosome IX. Genes influencing awning were associated with chromosomes VIII, IX and X. In a later study (54) he associated the *sphaerococcum* gene with chromosome XVI. He found that when this recessive gene was in the hemizygous condition, as was the case in the F_1's of the cross nullisomic XVI × *T. sphaerococcum*, the sphaerococcum character was not exhibited. Two doses of the gene, therefore, were needed for the development of this character. Sears and Rodenhiser (55) associated two complementary genes for resistance to stem rust with chromosome X of the variety Timstein. O'Mara (42), by using these deficiencies, was able to associate three major awn-inhibiting genes with chromosome VIII, IX and X. He concluded that the series of genes for awning in common wheat was now complete. Unrau and Swenson (68), in a preliminary report of the present study, showed the association of a number of genes with certain chromosomes.

Genetics of the Characters Involved in this Study

Glume Colour

Genetic studies have indicated red or brown glume colour to be dominant over white. Both monohybrid (13, 14, 18, 10, 60, 61, 62, 63, 73) and dihybrid (67) ratios have been obtained.

Spike Density

Apparently one main gene is responsible for differentiating "club" and "common" spikes in wheat (59, 19, 18, 60, 61, 62, 63, 65, 6). Most investigators also observed transgressive segregation in the material studied. Nilson-Ehle (39) explained such segregation with the hypothesis that there was a main gene, C, for club spikes epistatic to a series of L genes for length of rachis internode.

Awning

Lack of agreement in various reports on the genetics of awning probably arises out of the use of different classifications and material similar in phenotype but differing in genotype.

Tip-awned and fully awned varieties have been found to differ by one gene, with the fully awned condition recessive (38, 62, 4, 21, 20, 32, 67, 66, 46). Segregation appeared to be more complex in crosses involving completely awnless plants. In most cases (13, 4, 14, 11, 45) at least two gene pairs were involved with awn suppression dominant, though in one study (28) the results obtained were attributed to the operation of multiple genes. The most complete study by conventional genetic methods is that of Watkins and Ellerton (69). They postulated that if any two or all three of the genes B_2, B_1, or A_3 were in the homozygous dominant state, the plant would be awnless; if only one were homozygous dominant the plant would be tip-awned, while when all three were homozygous recessive the plant would be fully awned.

Spring vs. Winter Habit of Growth

Most workers have found spring habit of growth dominant, and simple ratios usually have been attained (16, 2, 61, 46). However, Powers (44) concluded that three pairs of genes of unequal effect controlled habit of growth and earliness. Aamodt (1), and Gaines and Singleton (20) found spring habit of growth dominant, but concluded that multiple genes were involved. Hayes and Aamodt (22), while attempting no factorial interpretation, found spring habit of growth and lack of winter hardiness dominant in crosses of Marquis with Minturki and Minhardi.

Reaction to Leaf Rust

Wells and Swenson (70) found a single partially dominant gene to be responsible for the resistance of H_{44}-Baringa. In a cross between two susceptible varieties, Swenson *et al.* (66) obtained a 9:7 ratio of resistant to susceptible plants in F_2. Maines *et al.* (35) concluded that one dominant gene pair was responsible for resistance of Malakoff under greenhouse conditions, while the field resistance of Kanred was dependent on several pairs of genes. In material involving H_{44} as one parent, Hayes *et al.* concluded that one or two pairs of genes were operating.

Reaction to Bunt

The resistance of the Martin gene to race T-1 has been found to be dominant by Briggs (9); but Smith (58) has found that the reaction varied depending on what race of the organism was used. Rodenhiser and Holton (47), and Holton (24) have studied and reported the reaction of a large number of varieties when infected with different races of the disease organism. A very complete review of the literature on the genetics of bunt has been published by Holton and Heald (25). Reference will be made later to specific related studies.

MATERIAL AND METHOD OF PROCEDURE

Description of Parent Varieties

Two varieties of *Triticum aestivum* L.*, Chinese Spring and Federation 41, and one of *T. compactum* Host., were used in this study. Chinese Spring, a variety of common wheat, was the source of the 17 nulli- or monosomic lines. These lines were obtained from E. R. Sears, and his system of numbering the deficiencies is used throughout the study.

Below is given a description of the varieties with respect to the characters which were studied:

Character	Variety		
	Chinese Spring *T. aestivum*	Federation 41 *T. aestivum*	Hymar *T. compactum*
Glume colour	White	Red	White
Habit of growth	Spring	Spring	Winter
Awning	Awnless	Awnless	Short awnlets
Reaction to leaf rust, *Puccinia rubigo-vera tritici* Eriks.	Mature plant resistance	Susceptible	Susceptible
Reaction to bunt *Tilletia caries* (D.C.) Tul., Race T-16	Susceptible	Resistant (Martin gene present)	Resistant (Martin gene present)
Spike density	Lax	Lax	Dense

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* Synonymous with *T. vulgare* Vill., until recently the commonly accepted botanical name of the species, although *T. aestivum* has priority since it was the original Linnean designation.

Experimental Methods

Seed of the 17 different deficient lines was planted in the spring of 1945. In lines I and VII, nullisomic plants were used as female parents for making the crosses. In two other lines, IX and XVIII, where monosomic plants could be distinguished from normal 2n plants, only monosomics were used. In the other lines, phenotypically normal (in most cases monosomic)* plants were used. Seed was harvested from all parent plants for progeny tests.

Because abnormal transmission of a gene carried by a particular chromosome will occur in F_2 families only if the F_1 plants are monosomic, it was necessary to determine the chromosomal constitution of the female parent plants in the 13 lines where monosomics were indistinguishable from the disomic plants. This information was obtained by: (1) progeny tests of parent plants to determine if nullisomics were present; (2) cytological observations on the parent plants; (3) cytological observation on F_2 families (made on all families of crosses with Federation 41). This latter study also made it possible to infer the chromosomal constitution of the F_1 plants.

Since in most cases the same plants were used as female parents for crosses with Federation 41 and Hymar, the results of cytological studies and progeny tests could also be applied to the Hymar crosses.

Glume colour was studied in F_2 and F_3 populations of the crosses of Federation 41 on the 17 deficient lines of Chinese Spring. The F_2 plants were classified as being red- or white-glumed; the F_3 lines as homozygous red-glumed, segregating for glume colour, or homozygous white-glumed. Cytological data were obtained on a number of F_3 lines and plants grown from abnormally segregating F_2 families.

F_2 and F_3 data on the genetics of spike density were obtained from the crosses of the 17 monosomic (or nullisomic) lines of Chinese Spring with Hymar. In classifying F_2 plants, three classes were used: (1) dense, when the spikes were as dense as or denser than spikes of Hymar; (2) lax, when the spikes were as lax as or laxer than spikes of Chinese Spring, and (3) intermediate, when the spikes were intermediate in density. F_3 lines were classified as dense, lax, or segregating. Cytological data were obtained on a number of F_3 plants grown from F_2 plants of abnormally segregating families.

Awning was studied in F_2 populations of the crosses of Hymar on the 17 different deficient lines of Chinese Spring. Four classes, depending on the extent of awn development, were used: (1) awnless; (2) awnletted, if short awnlets were present; (3) apically awned, if awns were present on the apex of the spikes, and (4) awned, when the plant was fully awned.

Habit of growth was studied in spring-planted F_2 and F_3 populations of the crosses of Hymar on the 17 deficient lines of Chinese Spring. At maturity of the spring segregates, F_2 plants (or plants in segregating F_3 lines) were classified as spring if they were headed, while those that had failed to show any evidence of jointing were classified as having winter habit of growth. F_3 lines were classified as having spring, segregating, or winter habit of growth.

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* About 75-85 per cent of the functional eggs from monosomic plants are n-1; hence most of the F_1 plants from monosomic female parent plants are monosomic (cf. Review of Literature).

Reaction to leaf rust was studied in crosses of Federation 41 on the 17 different deficient lines of Chinese Spring. F_2 data were obtained from special rust nurseries in 1947 and 1948. F_3 data also were obtained in 1948. In 1947 F_2 plants were classified as resistant, intermediate, or susceptible. In 1948 there was no distinguishable intermediate F_2 class. F_3 lines were classified as resistant, segregating, or susceptible.

Bunt reaction was studied in F_2 populations of crosses of the 17 Chinese Spring deficiencies with both Hymar and Federation 41. From each F_1 plant 100 to 150 seeds were inoculated with spores of race T-16* and space planted in a nursery at the Pendleton Field Station, Pendleton, Oregon, in the fall of 1946. A similar nursery from the crosses involving Federation 41 was planted at Pullman in the spring of 1947. Where F_3 data were needed, seeds from smut-free F_2 plants were inoculated and tested in the same way. At maturity F_2 plants were examined for smutted spikes and classified into four classes with respect to the presence of smut: (1) all spikes smutted; (2) most spikes of main tillers smutted; (3) few spikes smutted. and (4) smut-free. F_3 lines were classified as smut-free or segregating. For analysis of the data classes 1 and 2 were combined into the susceptible class, and classes 3 and 4 into the resistant class. For computing Chi-square, the data were corrected for escapes of the susceptible genotypes, The percentage of escapes of Chinese Spring was used as the basis for correction.

Cytological observations were made using the acetocarmine and acetoorcein smear techniques which have been described in greater detail by Smith (57). Whole spikes of the parent plants were collected in 1945, fixed in Carnoy's fluid for three to four days, and stored in 70 per cent alcohol at 5° to 10° C. until the summer of 1948. Spikes of F_2 plants were collected in the summer of 1947. They were fixed and stored in Carnoy's fluid until they were examined in 1948. Spikes of F_3 plants were fixed in Carnoy's fluid for three to four days, after which they were studied cytologically. Root-tips were pre-treated in a saturated solution of paradichlorobenzene for from five to six hours, fixed in Farmers' fluid for from two to three days, and stored in 70 per cent alcohol until used.

Observations on pollen mother cells were made on acetocarmine smear preparations. In most cases chromosome counts could be made at a magnification of about 400 diameters using a 40x high dry objective and a 10x ocular. In some cases, where meiosis had been completed and where it was necessary only to determine whether or not a plant was monosomic, the presence of micronuclei in a high percentage of the quartets was taken as evidence that the plant was monosomic. In most cases, however, actual counts of chromosomes were obtained. Root-tip examinations were on acetoorcein smear preparations.

Photomicrographs of microsporocytes were taken of acetocarmine smear slides made permanent by the tertiary-butyl alcohol method. Photomicrographs of root-tip cells were taken of freshly prepared slides.

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* Inoculum kindly supplied by C. S. Holton.

TABLE 1.—CHROMOSOMAL CONSTITUTION OF FEMALE PARENTS AND F_1'S OF MONOSOMIC (OR CORRESPONDING NULLISOMIC) LINES OF CHINESE SPRING × FEDERATION 41

Chromosome tested	Constitution of parents as indicated by			F_1 plants tested	Constitution of F_2 plants				Constitution of F_1 plants (inferred)	
	Phenotype	Progeny test	Cytologic observation		21^{II}	$20^{II}1^{I}$	20^{II}	Total	$20^{II}1^{I}$	21^{II}
				No.	No.	No.	No.	No.	No.	No.
I	2n-2	2n-2	—	6	3	12	—	15	6*	0
II	?	—	$20^{II}1^{I}$	—	—	—	—	—	—	—
	?	2n-1	—	3	4	3	0	7	2	1
	?	2n-1	—	3	2	6	0	8	3	0
III	?	2n-1	$20^{II}1^{I}$	3	5	5	0	10	2	1
	?	2n-1	$20^{II}1^{I}$	3	0	6	0	6	3	0
IV	?	?	$20^{II}1^{I}$	6	5	9	0	14	5	1
V	?	2n-1	$20^{II}1^{I}$	—	—	—	—	—	—	—
	?	2n-1	—	3	1	5	0	6	3	0
	?	2n-1	$20^{II}1^{I}$	3	4	4	0	8	2	1
VI	?	2n-1	$20^{II}1^{I}$	6	7	8	0	15	5	1
	?	2n	$20^{II}1^{I}$	—	—	—	—	—	—	—
	?	2n	$20^{II}1^{I}$	—	—	—	—	—	—	—
VII	2n-2	2n-2	20^{II}	6	3	16	0	19	6	0
VIII	?	2n-1	—	3	6	6	0	12	2	1
	?	2n-1	$20^{II}1^{I}$	2	4	4	0	8	1	1
IX	2n-1	2n-1	$20^{II}1^{I}$	3	0	5	0	5	3	0
	2n-1	2n	$20^{II}1^{I}$	—	—	—	—	—	—	—

X	?	2n	—	3	6	2	0	8	2	1
	?	2n	$20^{II}1^{I}$	3	4	3	0	7	2	1
XI	?	2n	$20^{II}1^{I}$	3	3	6	0	9	3	0
	?	2n	—	3	10	2	0	12	1	2
XV	?	2n-1	$20^{II}1^{I}$	3	0	7	0	7	3	0
	?	2n-1	$20^{II}1^{I}$	3	1	6	0	7	3	0
	?	2n	21^{II}	—	—	—	—	—	—	—
XVI	?	2n-1	—	3	1	4	0	5	3	0
	?	2n-1	$20^{II}1^{I}$	3	5	4	0	9	2	1
XVII	?	2n	—	3	5	3	0	8	2	1
	?	2n	—	3	8	3	1	12	1	2
	?	2n	—	3	5	2	2	9	2	1
XVIII	2n-1	2n-1	$20^{II}1^{I}$	6	6	12	0	18	5	1
XIX	?	2n-1	$20^{II}1^{I}$	6	5	19	0	24	5	1
XX	?	2n-1	$20^{II}1^{I}$	3	4	5	0	9	2	1

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[*] If the first plant in an F_2 family was found to be monosomic, usually no more plants were examined

EXPERIMENTAL RESULTS

Chromosomal Constitution of Female Parents and of F_1's from Monosomic (or Corresponding Nullisomic) Lines of Chinese Spring × Federation 41

The results of progeny tests and cytological studies of the female parent plants, as well as determinations of the chromosomal constitution of the F_1's from cytological studies on F_2 plants are presented in Table 1.

In 10 cases the chromosomal constitution of the female parent plants was not shown by the progeny test. This may be explained on the basis of: (1) small populations; (2) low transmission of n-1 male gametes, or (3) low survival of nullisomic plants. In the case of all 17 lines tested, however, at least some of the F_1 plants were monosomic as shown by cytological studies on F_2 plants. Thus, data on the effect of each of the 17 chromosome deficiencies on the transmission of the genes for the several characters were obtained.

Chromosomal Constitution of Female Parents and of F_1's from Monosomic (or Corresponding Nullisomic) Lines of Chinese Spring × Hymar

As indicated earlier, the female parent plants used for making these crosses, in most cases, were the same as those used in the Federation 41 crosses. It is assumed, therefore, that in these crosses the F_2 families representing the 17 different chromosome deficiencies also were from monosomic F_1 plants.

Glume Colour

The data on segregation of glume colour in F_2 and F_3 populations from crosses of Federation 41 on the 17 different monosomic (or corresponding nullisomic) lines of Chinese Spring are summarized in Table 2.

Excepting for families involving chromosome I, the F_2 results clearly indicate that a single dominant gene pair was responsible for red glumes in Federation 41. These results are substantiated by the close fit to a 1 : 2 : 1 ratio of homozygous red-glumed : segregating : homozygous white-glumed lines in the 139 F_3 lines tested.

The unsatisfactory fit obtained when the results of all F_2 families, excluding those involving chromosome I, are combined probably can be attributed to errors in classification of some white-glumed plants. Since these same populations were used for studying bunt reaction and since smut causes glume discoloration, it is likely that some genetically white-glumed plants were classified as having red glumes. This error would be consistent and the deviation would be cumulative, resulting in a highly significant X^2 when the data are combined.

Segregation for glume colour in the chromosome I families was obviously different from that in other families. The proportion of white-glumed plants was abnormally low, and judged by their lack of vigour and fertility, all of these white-glumed plants were nullisomic. This indicates conclusively that the gene for red glumes of Federation 41 is carried by chromosome I.

In Figure 1, spikes from red- and white-glumed (presumably nullisomic) F_2 plants of families involving chromosome I are illustrated.

TABLE 2.—SEGREGATION FOR GLUME COLOUR IN F_2 AND F_3 POPULATIONS FROM CROSSES OF FEDERATION 41 ON THE 17 DIFFERENT MONOSOMIC (OR CORRESPONDING NULLISOMIC) LINES OF CHINESE SPRING

Chromosome tested	Red-glumed	Segregating	White-glumed	Total	X^2 for ratio of 3 : 1 in F_2 and 1 : 2 : 1 in F_3
	No.	No.	No.	No.	
I	528	—	38	566	100.93**
II	637	—	193	830	1.35
III	628	—	219	847	0.33
IV	600	—	196	796	0.06
V	652	—	202	854	0.83
VI	748	—	228	976	1.45
VII	659	—	208	867	0.47
VIII	690	—	213	903	0.96
IX	248	—	73	321	0.87
X	797	—	248	1045	0.89
XI	915	—	292	1207	0.42
XV	708	—	224	932	0.47
XVI	814	—	264	1078	0.15
XVII	1308	—	417	1725	0.63
XVIII	578	—	174	752	1.39
XIX	679	—	212	891	0.69
XX	314	—	99	413	0.23
Total (excluding families involving chromosome I)	10,975	—	3,462	14,437	8.00**
F_3 lines*	33	70	36	139	0.12

* The F_3 data on normal segregation were obtained from families segregating monosome XVI, but could have been obtained from any F_3 families other than those segregating monosome I.

** Significant at the 1 per cent level.

Because the F_2 results had indicated that the gene for red glume colour was carried by chromosome I, an effort was made to correlate genetic with cytologic observations. Microsporogenesis was studied in plants of a number of F_3 families grown from red- and white-glumed F_2 plants in families involving chromosome I. At maturity, the glume colour of plants studied cytologically was recorded. These data are summarized in Table 3.

TABLE 3.—CHROMOSOMAL CONSTITUTION AND GLUME COLOUR OF F_3 PLANTS FROM RED- AND WHITE-GLUMED F_2 PLANTS OF THE CROSS NULLISOMIC I × FEDERATION 41

F_2 parent plants			F_3 plants						
Glume colour	Chromosome* constitution	Total studied	21^{II}		$20^{II}1^{I}$		20^{II}		Total F_3 plants studied
			Red	White	Red	White	Red	White	
		No.	No.	No.	No.	No.	No.	No.	No.
Red	2n	14	53	0	0	0	0	0	53
Red	2n-1	10	18	0	119	0	0	0	137
White	2n-2	6	0	0	26	11	0	0	37
Total		30	71	0	145	11	0	0	227

* Inferred from observations on F_3 plants.

As expected, no white-glumed 2n or 2n-1 F_3 plants were present in the progenies of red-glumed F_2 plants. It was surprising, however, that not a single 2n-2 plant was found in any of the families that segregated monosomics. This may have been because there was a severe infestation of the wheat stem maggot* in the nursery. Nullisomics, being weak and producing few culms even under ideal conditions, were possibly unable to survive attack by the parasites.

There was no apparent difference in glume colour of disomic and monosomic F_3 plants. Thus, one gene was adequate for developing full red glume colour. In Figure 2 a microsporocyte and a spike from a monosomic F_3 plant are illustrated.

The F_3 results from white-glumed (presumably nullisomic) F_2 plants require some explanation. Obviously selfed progeny from them should be like the parents, i.e., white-glumed and nullisomic. The F_2 plants were allowed to open-pollinate, and consequently a high percentage (or possibly all) of their progeny resulted from natural crosses, since nullisomic I plants are almost completely male sterile. At any rate, the 26 red-glumed F_3 monosomic plants must have resulted from natural crossing.

The assumption that the white-glumed F_2 plants were nullisomic and cross pollinated is further supported by the fact that all progenies examined cytologically were 2n-1.

Spike Density

The data on segregation of spike types in F_2 and F_3 populations of the crosses of Hymar on the 17 different monosomic (or corresponding nullisomic) lines of Chinese Spring are summarized in Table 4.

In F_2 families of chromosomes not associated with the gene conditioning spike density, a simple monohybrid ratio was expected. However, the expected ratios were not obtained, probably because of erroneous classification of a number of F_2 plants. The same families were used also in the study of bunt reaction, and probably some smutted plants were wrongly classified, since the diseased spikes were abnormally elongated.

Segregation in F_2 populations indicated that a single gene pair was mainly responsible for differentiation between the true lax and the true dense spike types. However, there was evidence of transgressive segregation, some plants having spikes more dense than Hymar, and others having spikes more lax than Chinese Spring (Figure 3). This indicated that there were other (possibly only modifying) genes which affected density of spikes.

Segregation in F_2 families involving chromosome XX was distinctly different from that in families in which the other 16 chromosomes were tested. There were only 11 lax-spiked F_2 plants. The lack of vigour and the low fertility of these plants indicated that they were probably nullisomic (Figure 4A). These results suggested that the main gene for dense spikes in Hymar was carried by chromosome XX.

F_3 data obtained from 112 F_2 plants were not entirely as expected. In 29 of the F_3 progenies there were 51 lax-spiked plants; 10 of the 51 were similar to the 11 presumably nullisomic F_2 plants, the remaining 41 plants had normal vigour and fertility, and at maturity 19 of the 41 were found

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* Larvae of the insect were present in approximately half of the plants examined cytologically.

FIG. 1

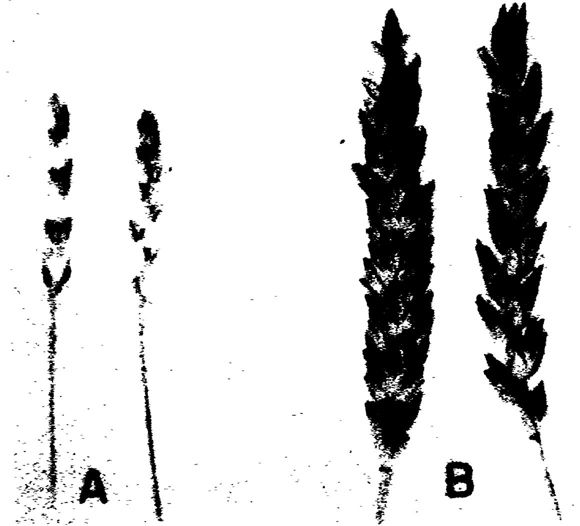


FIGURE 1. *(left)* A. Spikes from white-glumed, apparently Nullisomic I, F_2 plants.
B. Spikes from red-glumed, normal or monosomic I, F_2 plants.

FIGURE 2. *(lower left)* A. A microsporocyte from a 2n-1 F_3 plant of nullisomic I × Federation 41. There are 13 closed pairs, seven open pairs, one univalent (arrow).
B. A spike from the same plant as Figure 2A. The glumes were red and indistinguishable from 2n plants.

FIGURE 3. *(lower right)* Spike and rachis types of parents, and segregates from crosses of Hymar on the 17 different deficient lines of Chinese Spring.
A. Hymar.
B. and C. F_3 segregates.
D. Chinese Spring.

FIG. 2

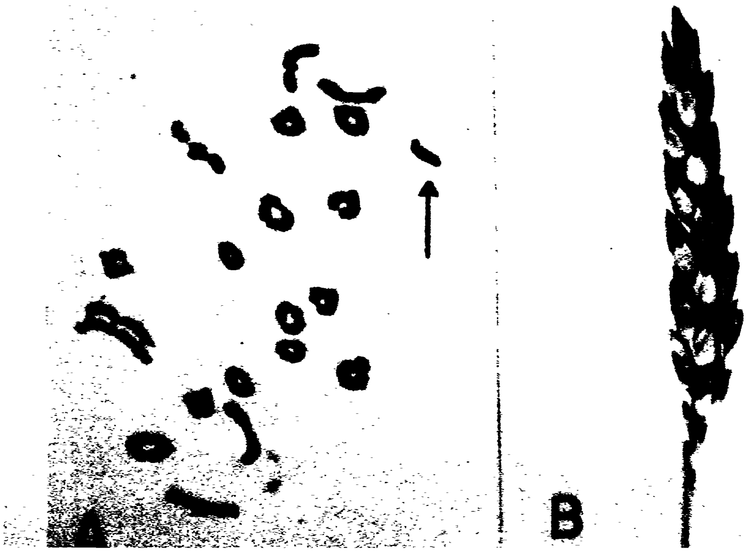


FIG. 3

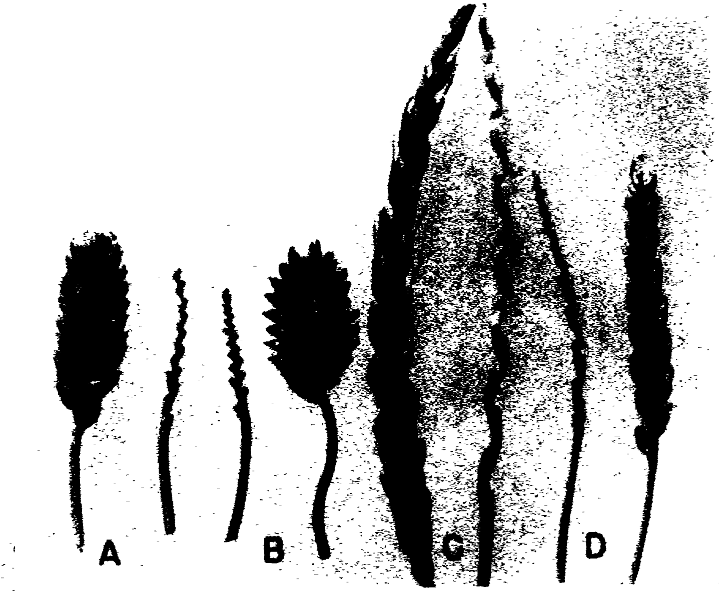


FIGURE 5. Awn types of parent and F_2 plants of the crosses of Hymar on the 17 different monosomic (or corresponding nullisomic) lines of Chinese Spring.

A. Spikes from fully awned F_2 plants.

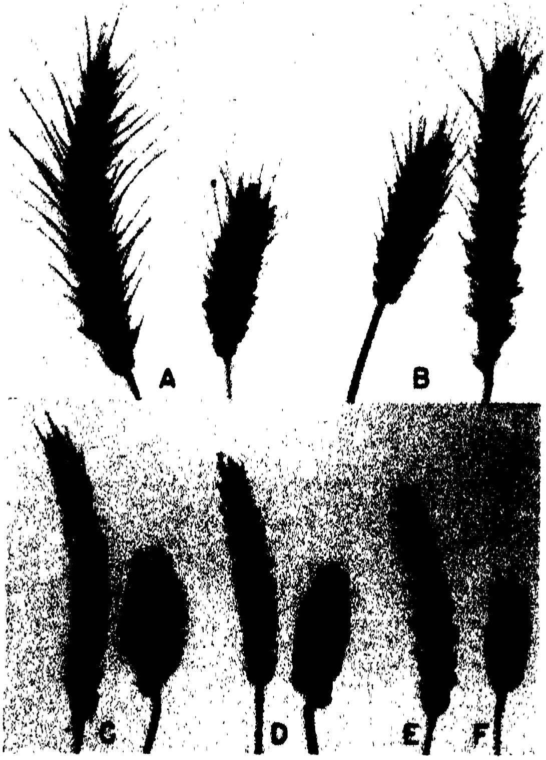
B. Spikes from apically awned F_2 plants.

C. Spikes from awnletted F_2 plants.

D. Spikes from awnless F_2 plants.

E. Spike from Chinese Spring.

F. Spike from Hymar.



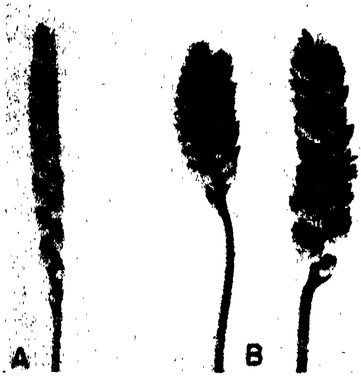


FIGURE 4. Spikes from F_2 plants of the cross of Hymar on Chinese Spring deficient for chromosome XX.

A. Spike from plant presumed to have 2n-2 chromosomes.

B. Spikes from plants presumed to have 2n or 2n-1 chromosomes.

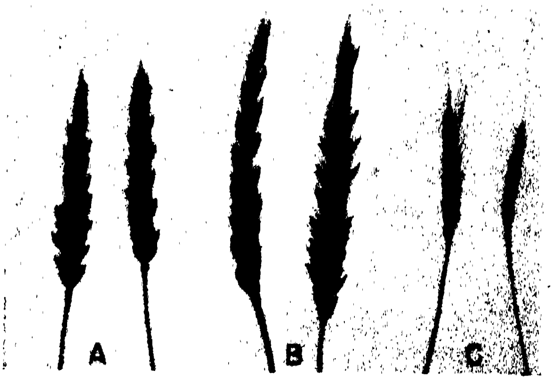


FIGURE 6. Spikes from F_2 plants of the cross of Hymar × monosomic IX of Chinese Spring.

A. and B. Spikes from monosomic (heterozygous speltoid) plants.

C. Spike from a plant that was presumably nullisomic (homozygous speltoid).

TABLE 4.—SEGREGATION OF SPIKE TYPES IN F_2 AND F_3 POPULATIONS OF THE CROSSES OF HYMAR ON THE 17 MONOSOMIC (OR CORRESPONDING NULLISOMIC) LINES OF CHINESE SPRING

	Spike type of plants (lines in F_3)				
Chromosome tested	Dense	Intermediate (or segregating in F_3)	Lax	Total	X^2 for 1 : 2 : 1 ratio
	No.	No.	No.	No.	
I	57	98	78	233	—*
II	143	125	119	387	—
III	117	113	87	317	—
IV	62	67	53	182	—
V	119	97	95	311	—
VI	90	85	89	264	—
VII	60	54	47	161	—
VIII	174	175	157	506	—
IX	96	140	142	378	—
X	166	199	158	543	—
XI	195	187	149	531	—
XV	104	112	88	304	—
XVI	134	108	102	344	—
XVII	107	91	91	289	—
XVIII	141	139	122	402	—
XIX	35	52	47	134	—
XX	154	66	11	231	—
Total (excluding families involving chromosome XX)	1820	1842	1624	5286	—
F_3 lines**	124	249	118	491	0.24

* The F_2 data could not be fitted to a 1 : 2 : 1 ratio, so X^2 was not computed.

** These data on normal segregation were obtained from families segregating monosomes IX and X, but could have been obtained from any families other than those segregating monosome XX. Since the segregation in families involving the monosomes IX and X was similar, the data are combined.

to be red-glumed. This suggests that probably all 41 lax-spiked plants with normal vigour were natural crosses; certainly the red-glumed ones were, since Hymar and Chinese Spring both have white glumes.

The cytological data on F_3 lines obtained from F_2 populations involving chromosome XX are summarized in Table 5.

While no nullisomic plants were found among the 71 plants studied, a high proportion of the plants had aberrations other than univalents. These aberrations involved chains of three or four chromosomes, heteromorphic bivalents, pairs of telocentric chromosomes and isochromosomes. All plants with the normal chromosomal complement had dense spikes, while those with 20 pairs of chromosomes plus one univalent or 20 pairs plus one heteromorphic bivalent, had spikes of intermediate density. The fact that plants with 20 pairs plus one heteromorphic bivalent had spikes of intermediate density indicates that the Hymar gene for spike density was not present in the telocentric chromosome. It can further be inferred that the telocentric chromosome was present in the plant used as the female parent in making the crosses, since the aberration was present in F_3 lines obtained from four different F_2 families.

It is likely that the chains of three or four chromosomes were the result of supernumerary chromosomes rather than reciprocal translocations. The one plant with a chain of four chromosomes (cf. Table 5) had in addition

TABLE 5.—CHROMOSOMAL CONSTITUTION AND SPIKE TYPE OF F_3 PLANTS OF THE CROSS OF HYMAR ON A LINE OF CHINESE SPRING WHICH WAS DEFICIENT FOR CHROMOSOME XX

Total chromosomes*	Chromosomal constitution	Spike type	F_3 plants studied	F_3 families represented
No.			No.	No.
42	21^{II}	Dense	21	10
41	$20^{II}1^{I}$	Intermediate	13	5
42	$20^{II}1^{I}$ heteromorph. pr.	Intermediate	23	7
44	22^{II}	Dense	1	1
44	$20^{II}2^{I}$ 1 het. pr.	Lax, normal	1	1
43	19^{II} 1 telo. pr. 1^{III}	Intermediate	1	1
44	20^{II} 1^{IV}	(Died)	1	1
41	20^{II} 1 iso.	Int. to near lax	3	1
43	19^{II} 1 iso. 1^{III}	Int. to near lax	1	1
42	$19^{II}2^{I}$ 1 het. pr.	Intermediate	3	1
43	20^{II} 1^{I} 1 het. pr.	Dense	1	1
44	21^{II} 1 telo. pr.	Dense	1	1
43	$20^{II}1^{I}$ 1 telo. pr.	Dense	1	1

* Includes also half chromosomes and isochromosomes.

20 normal pairs of chromosomes. One possible source of plants with such a chromosomal constitution would be plants with two or more univalents, or with chains of three chromosomes (Sears 50, 52).

The cytological studies showed that the F_2 and F_3 plants with lax spikes, and which were classified as nullisomic, had a pair of telocentric chromosomes (Plate I, I). The root-tip cell illustrated in Plate I, J is from one of the 10 F_3 plants that were classified as nullisomic (spike illustrated in Plate II G). This plant had 40 normal and two half (presumably telocentric) chromosomes, and it is probable that this was the chromosomal constitution of all F_2 and F_3 plants in this family that were classified as nullisomic.

PLATE I

Microsporocyte and root-tip cells from F_3 plants of the cross of Hymar on Chinese Spring, deficient for chromosome XX.

A. Microsporocyte from a disomic plant, 21 pairs.

B. Microsporocyte from a monosomic plant, 18 closed pairs, 2 open pairs, 1 univalent (arrow).

C. Microsporocyte from a plant with 18 closed pairs, 1 open pair, 2 univalents (8 and 9 o'clock), 1 heteromorphic bivalent (arrow).

D. Microsporocyte from a plant with 19 closed pairs, 1 disjoined pair, 1 chain of 4 (arrow), 2n = 44.

E. Microsporocyte from the same floret as D, chain of 4 chromosomes (arrow).

F. Microsporocyte showing 1 heteromorphic bivalent at diakinesis (arrow). There also is evidence of a multiple association in the centre of the group.

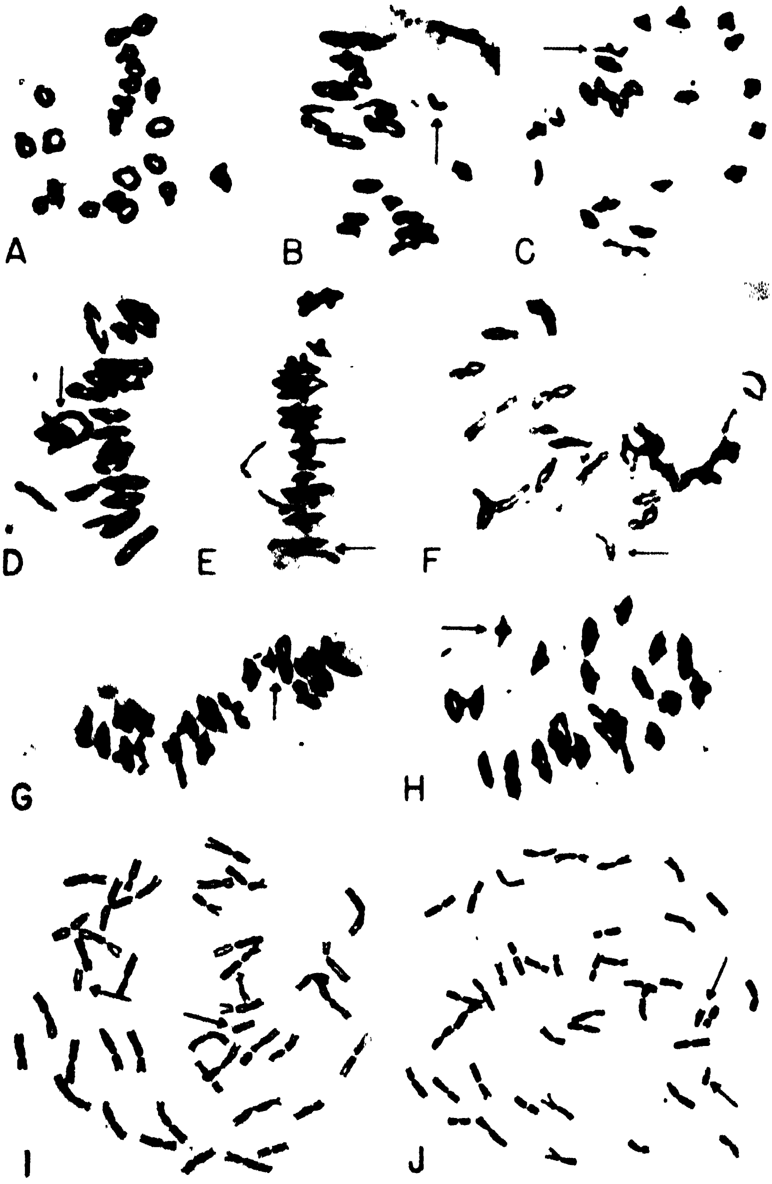
G. Microsporocyte from a plant with 1 (presumably) telocentric chromosome pair (arrow), 18 closed pairs, 1 chain of 3 chromosomes (bottom centre).

H. Microsporocyte from a plant of the same F_3 line as G, 20 closed pairs, 1 (presumably) telocentric pair (arrow), 1 open pair.

I. Root-tip metaphase from a progeny of plant G, 40 normal and 2 half (presumably telocentric) chromosomes (arrow).

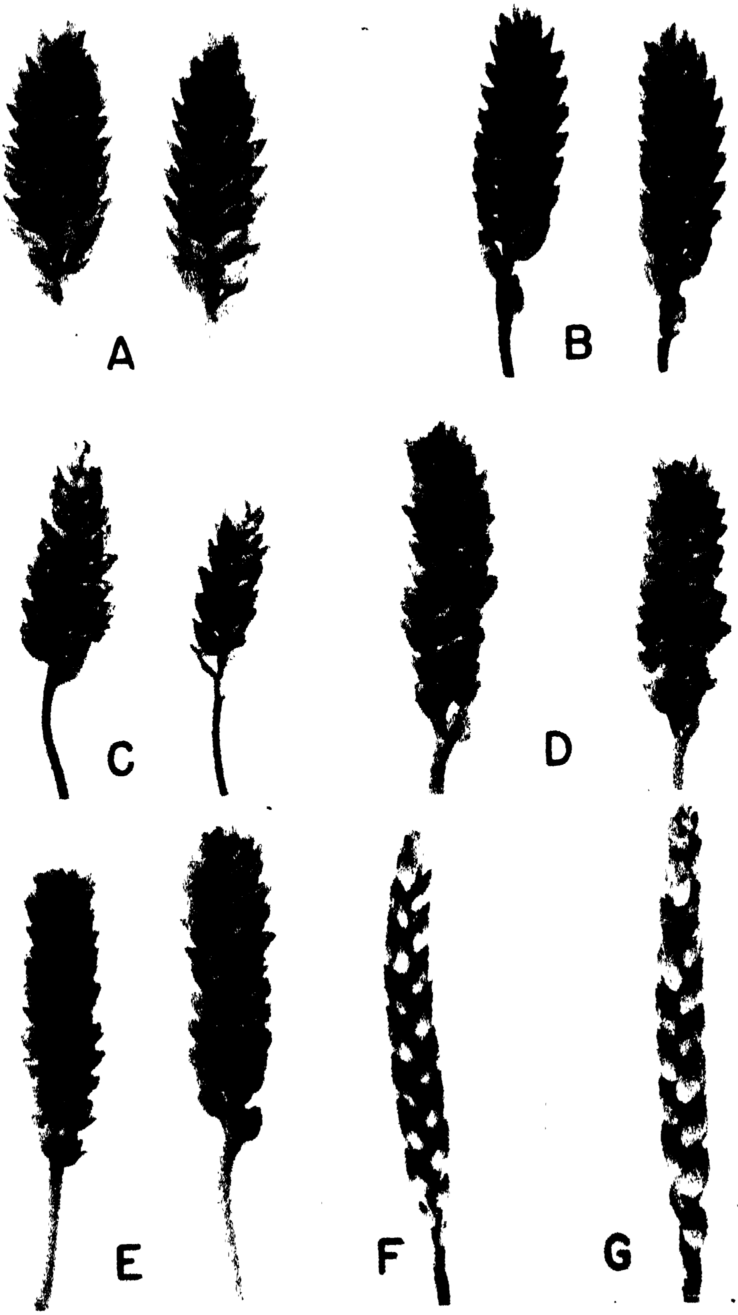
J. Root-tip metaphase from a progeny of an F_2 plant presumed to be nullisomic. There appear to be 40 normal chromosomes and 2 half (presumably telocentric) chromosomes (arrow).

(Magnifications of all photomicrographs approximately 1080x).



(Legend on page 78)

PLATE II



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Awning

The data on segregation of awn types in F_2 families of the crosses of Hymar on the 17 different monosomic (or corresponding nullisomic) lines of Chinese Spring are summarized in Table 6.

TABLE 6.—SEGREGATION OF AWN TYPES IN F_2 POPULATIONS FROM CROSSES OF HYMAR ON THE 17 DIFFERENT MONOSOMIC (OR CORRESPONDING NULLISOMIC) LINES OF CHINESE SPRING

Chromosome tested	Awnless and awnletted	Apically awned and awned	Total	X^2 for ratio of 57 : 7
	No.	No.	No.	
I	26	2	28	0.35
II	159	16	175	0.56
III	51	11	62	2.98
IV	57	11	68	1.96
V	61	7	68	0.04
VI	83	10	93	<0.01
VII	31	4	35	<0.01
VIII	227	56	283	23.76*
IX	137	0	137	16.84*
X	181	40	221	11.68*
XI	215	23	238	0.39
XV	148	19	167	<0.01
XVI	151	18	169	<0.01
XVII	158	15	173	0.89
XVIII	152	13	165	1.56
XIX	95	11	106	0.01
XX	177	13	190	3.28
Total F_2 (excluding families involving chromosomes VIII, IX, and X).	1564	173	1737	1.70

* Significant at the 1 per cent level.

Although Chinese Spring is awnless, and Hymar has only very short awnlets, fully awned, awnless and various intermediate types were observed in F_2 populations, as illustrated in Figure 5. According to Sears (50), Chinese Spring has one dominant awn inhibiting gene in chromosome VIII and another in chromosome X. Evidently Hymar had the recessive alleles in these same chromosomes, but also a dominant awn-inhibiting gene in a

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PLATE II

Spikes from cytologically examined F_3 plants of the cross of Hymar on Chinese Spring, deficient for chromosome XX.

A. Spike from a 2n plant classified as dense.
B. Spike from a 2n-1 plant classified as intermediate.
C. Spike from a plant with 1 chain of 4 and 20 pairs of chromosomes.
D. Spike from a plant with 1 heteromorphic bivalent (from same plant as microsporocyte in Plate I, F).
E. Spike from a plant with 41 normal chromosomes and 1 (presumably) telocentric pair (from same plant as microsporocyte in Plate I, G).
F. Spike from a plant with 40 normal and 2 half (presumably) telocentric chromosomes. This plant was from progeny of the plant illustrated in Plate II, E.
G. Spike from a plant classified as nullisomic. (Microsporocyte from a progeny of this plant illustrated in Plate I, J)

(Magnification approximately 1x).

chromosome other than VIII and X. Consequently, a trihybrid ratio would be expected in the F_1 families involving chromosomes not associated with genes for awns. If the awnless and awnletted plants are combined into one class and apically awned and fully awned plants also are grouped, and further, if it is assumed that plants with two or more dominant awn inhibitor genes were awnless or awnletted, the expected F_2 ratio would be 57 awnless and awnletted : 7 apically awned and awned plants.

With the exception of families involving chromosomes VIII, IX, and X, the results obtained (taken separately or collectively) were in fairly good agreement with the expected ratio, although some of the populations were small. The distribution of awn types was distinctly different in families segregating monosomes VIII, IX, and X from that in families involving the other 14 chromosomes.

In families involving chromosomes VIII and X, there was a much higher proportion of apically-awned and fully awned plants than in families involving the other chromosomes tested. This would be expected if the recessive alleles of the two awn-inhibiting genes of Chinese Spring are carried by chromosomes VIII and X of Hymar. The ratio in families involving these two chromosomes closely approached 50 awnless and awnletted: 14 apically awned and fully awned, the X^2 being 0.005 and 0.72 for families involving chromosomes VIII and X, respectively. Such a ratio would result if in families involving these chromosomes, the monosomic plants that were heterozygous for the Hymar awn-inhibiting gene were awnletted. In Table 7 hypothetical genotypes and phenotypes for families involving these two chromosomes are suggested. Since there were

TABLE 7.—POSTULATED F_2 PHENOTYPES AND GENOTYPES OF CROSSES OF HYMAR ON MONOSOMIC (OR NULLISOMIC) LINES VIII AND X OF CHINESE SPRING

Awnless and awnletted classes		Apically awned and awned classes	
Genotype	Proportion of 64	Genotype	Proportion of 64
	No.		No.
a a $A_2A_2A_3A_3$*	1	a a $A_2a_2a_3a_3$	2
– a $A_2A_2A_3A_3$**	3	a a $a_2a_2A_3a_3$	2
a a $A_2A_2a_3a_3$	1	– a $a_2a_2A_3a_3$	6
– a $A_2A_2a_3a_3$	3	a a $a_2a_2a_3a_3$	1
a a $A_2a_2A_3A_3$	2	– a $a_2a_2a_3a_3$	3
– a $A_2a_2A_3A_3$	6		
a a $A_2a_2A_3a_3$	4		
– a $A_2a_2A_3a_3$	12		
a a $A_2A_2A_3a_3$	2		
– a $A_2A_2A_3a_3$	6		
a a $a_2a_2A_3A_3$	1		
– a $a_2a_2A_3A_3$	3		
– a $A_2a_2a_3a_3$	6		
Total	50		14

* A was suggested as the symbol for genes of awns by the committee on nomenclature and genetic factors in wheat (5). It is here suggested that A without subscript and A_2 and A_3 be used to denote the awning genes in chromosomes VIII, IX, and X, respectively.

** – Indicates the absence of a gene in monosomic plants. The table suggests genotypes for families segregating monosomic VIII plants. For families where chromosome X was involved the same proportions would be obtained, but A_3 would be affected.

only a few nullisomics, they have been omitted entirely. It also was assumed that monosomic and disomic plants were present in a proportion of 75 : 25, respectively (cf. Review of Literature).

The validity of including the – a $A_2a_2a_3a_3$ genotype in the awnless and awnletted class is questionable. This can be justified only if A_2, the Hymar awn inhibiting gene, has greater effect than either of the Chinese Spring genes A and A_3. Results obtained in families segregating monosomic IX plants indicated that this might be a valid assumption.

Segregation for awns in families involving the chromosome IX deficient line of Chinese Spring was distinctly different from that in families involving all the other chromosomes. At the most, disomic and monosomic plants were awnletted, and only the occasional nullisomic plant had appreciable awn development (Figure 6). This indicates that the dominant awn inhibiting gene of Hymar was carried by chromosome IX. The fact that none of the monosomic plants (easily identified since they were speltoid) was apically-awned is evidence of the correctness of the conclusion reached earlier; namely, that the Hymar gene has greater effect on inhibiting awn development than either of the two genes in Chinese Spring.

Spring vs. Winter Habit of Growth

The data on segregation for growth habit in F_2 and F_3 populations from the crosses of Hymar on the 17 deficient lines of Chinese Spring are summarized in Table 8.

The F_2 results, with the exception of those from chromosome IX families, closely fit a ratio of 15 spring: 1 winter, indicating that duplicate genes conditioned habit of growth in these crosses. While a significant X^2 was obtained in chromosome I and chromosome XX families, the results were close to the expected 15 : 1 ratio. The combined data also showed a very good fit to the duplicate gene F_2 ratio. Finally, this hypothesis was satisfactorily corroborated by the data from F_3 lines where a good fit to the expected ratio of 7 homozygous spring: 8 segregating: 1 homozygous winter line was obtained.

Segregation in F_2 and F_3 populations of chromosome IX was distinctly different from that observed in the other families. Actually, the data from F_2 populations combined with those from segregating F_3 lines involving chromosome IX give a very good fit to a 3 : 1 ratio. If one of the duplicate genes was carried by chromosome IX, this would be the ratio expected, since only the 1 dominant gene for spring growth habit of Chinese Spring (not carried by chromosome IX) would be segregating in these families. F_3 lines obtained from spring planted F_2 progenies would consist of one spring: two segregating, which was approximately the ratio obtained. There would be no true-breeding winter F_3 lines since no F_2 plants with the genotype for winter growth habit would have produced seed.

These results, therefore, clearly show that one of the duplicate genes conditioning habit of growth in these crosses was carried by chromosome IX.

Reaction of Leaf Rust

The data on leaf rust reaction of F_2 plants and F_3 lines from the crosses of Federation 41 on the 17 different monosomic (or corresponding nullisomic) lines of Chinese Spring are summarized in Table 9.

TABLE 8.—SEGREGATION FOR GROWTH HABIT IN F$_2$ AND F$_3$ POPULATIONS FROM THE CROSSES OF HYMAR ON THE 17 DIFFERENT MONOSOMIC (OR CORRESPONDING NULLISOMIC) LINES OF CHINESE SPRING

Chromosome tested	Growth habit of plants or lines			Total	Ratio expected	X^2
	Spring	Segregating	Winter			
I	432	—	16	448	15 : 1	5.48†
II	538	—	36	574	15 : 1	0.01
III	591	—	36	637	15 : 1	0.28
IV	236	—	18	254	15 : 1	0.33
V	499	—	29	528	15 : 1	0.51
VI	520	—	35	555	15 : 1	0.03
VII	290	—	12	302	15 : 1	2.63
VIII	457	—	33	490	15 : 1	0.20
IX*	3416	—	1107	4522	15 : 1	Very large††
IX**	365	—	28	393	15 : 1	0.51
X	944	—	68	1012	15 : 1	0.38
XI	716	—	44	760	15 : 1	0.28
XV	461	—	37	498	15 : 1	1.18
XVI	571	—	38	609	15 : 1	0.01
XVII	342	—	18	360	15 : 1	0.96
XVIII	784	—	49	833	15 : 1	0.19
XIX	42	—	5	47	15 : 1	1.51
XX	52	—	8	60	15 : 1	5.14†
Total F$_2$ (excluding families involving chromosome IX)	7840	—	510	8350	15 : 1	0.29
F$_3$ lines***	51	54	7	112	7 : 8 : 1	0.15
IX F$_3$ lines	93	144	0	237	1 : 2	3.72

* Combined data from F$_2$ populations and from segregating F$_3$ lines.
** F$_2$ data from disomic F$_1$'s.
*** The F$_3$ data on normal segregation were obtained from fall-planted families segregating monosome X, but could have been obtained from any F$_2$ families other than those segregating monosome IX.
† Significant at the 5 per cent level.
†† Significant at the 1 per cent level.

Segregation in F$_2$ families in 1947, with the exception of families involving chromosome IV, closely approached a ratio of one resistant: two intermediate: one susceptible, indicating that a single gene pair was determining the reaction. While the population in which chromosome IV was tested was small, the results indicated that the gene conditioning reaction to leaf rust was carried by this chromosome. If that were the case, the gene for resistance in Chinese Spring should have been entirely absent, and the three plants classified as resistant in this family could have been escapes.

The F$_2$ families and F$_3$ lines which were used for testing chromosome IV in 1948, did not, however, substantiate the 1947 findings. Actually, both generations gave normal monohybrid segregation in 1948.

Reaction to Bunt, Race T-16

The data on reaction to bunt, Race T-16, in F$_2$ and F$_3$ populations of the crosses of the 17 deficient lines of Chinese Spring with Federation 41, and in F$_2$ populations of the crosses with Hymar are summarized in Table 10.

In crosses of Hymar, a 3 : 1 ratio of resistant to susceptible plants was obtained in F$_2$ families involving all 17 chromosomes tested. If the Martin gene for resistance had been carried by one of the 17 chromosomes tested,

TABLE 9.—SEGREGATION FOR LEAF RUST REACTION IN F_2 AND F_3 POPULATIONS OF CROSSES OF FEDERATION 41 ON THE 17 DIFFERENT MONOSOMIC (OR CORRESPONDING NULLISOMIC) LINES OF CHINESE SPRING

Chromosome tested	Reaction of plants or lines			Total	χ^2 for ratio of 1 : 2 : 1
	Resistant	Intermediate (F_2) Segregating (F_3)	Susceptible		
	No.	No.	No.	No.	
I	73	152	74	299	0.08
II	97	170	82	349	1.53
III	68	157	84	309	1.73
IV	3	23	24	50	18.89†
IV*	77	—	193	270	1.78**
V	68	152	71	291	0.64
VI	47	105	64	216	2.84
VII	53	104	56	213	0.20
VIII	57	108	55	220	0.11
IX	24	41	22	87	0.37
X	56	137	69	262	1.84
XI	76	134	59	269	1.76
XV	54	131	64	249	1.76
XVI	59	113	56	228	0.01
XVII	55	131	62	248	1.19
XVIII	53	93	42	188	1.30
XIX	49	98	54	201	0.37
XX	15	25	10	50	1.00
Total F_2 (excluding families involving chromosome IV)	904	1851	924	3679	0.37
IV***	20	38	21	79	0.17

* Data from 1948. In this year the infection was more severe than in 1947 and no intermediate F_2 condition could be recognized.

** Ratio of 1 : 3 instead of 1 : 2 : 1.

*** F_3 lines instead of F_2 plants.

† Significant at the 1 per cent level.

except for the nullisomics, the entire F_2 family of the particular chromosome would have been resistant.

Segregation for reaction to bunt in F_2 populations derived from crosses of the 17 deficient lines of Chinese Spring with Federation 41 was different from that in populations of the Hymar crosses. With the exception of families involving chromosome XVI, segregation closely approached a ratio of 11 resistant: five susceptible plants. In families involving chromosome XVI the ratio obtained was as would be expected if the Martin gene alone were segregating. These results indicate that in the Federation 41 crosses a gene with a modifying effect on the Martin gene was present. This gene was associated with chromosome XVI as indicated by F_2 and F_3 data.

DISCUSSION

Conventional genetic methods have resulted in very slow and limited progress in developing linkage maps of chromosomes in common wheat. The difficulties encountered in attempting such studies probably stem largely from the fact that the species is polyploid, and from the paucity of easily classifiable characters.

TABLE 10.—SEGREGATION FOR REACTION TO BUNT, RACE T-16, IN F_2 AND F_3 POPULATIONS OF CROSSES OF FEDERATION 41, AND F_2 POPULATIONS OF THE CROSSES OF HYMAR ON THE 17 DIFFERENT MONOSOMIC (OR CORRESPONDING NULLISOMIC) LINES OF CHINESE SPRING

Chromosome tested	Federation 41 crosses				Hymar crosses			
	Reaction of plants (lines in F_3)			X^2 for † 3 : 1 F_2 or 1 : 2 F_3 ratio	Reaction of plants			X^2 3 : 1 F_2 ratio
	Resistant	Susceptible (Segregating in F_3)	Total		Resistant	Susceptible	Total	
	No.	No.	No.		No.	No.	No.	
I	431	190	621	34.00**	52	14	66	0.03
II	605	222	827	14.80**	185	40	225	2.24
III	634	235	869	17.24**	204	62	266	0.40
IV	565	236	801	33.59**	93	34	127	2.05
V	591	240	831	29.77**	168	53	221	0.77
VI	693	275	968	30.17**	184	68	252	4.76*
VII	635	246	881	24.04**	60	18	78	0.08
VIII	648	252	900	24.77**	391	121	512	1.26
IX	225	96	321	14.95**	460	136	596	0.44
X	781	318	1099	40.14**	310	107	417	4.48
XI	869	322	1191	23.44**	303	99	402	2.32
XV	668	248	916	18.28**	239	78	317	1.84
XVI	485	124	609	0.83	202	68	270	2.24
XVII	1175	530	1705	93.56**	275	81	356	0.25
XVIII	551	202	753	13.68**	125	40	165	0.77
XIX	645	253	898	26.12**	159	46	205	0.07
XX	310	143	453	30.76**	152	37	189	0.64
Total F_2 (excluding families involving chromosome XVI)	10,026	4008	14,034	459.03**	3562	1102	4664	11.56**
XVI††	26	54	80	0.05				

† Chinese Spring had 13 per cent escapes. This figure was used as a correction factor. Chi-squares were computed on the corrected results.

†† F_3 lines instead of F_2 plants. No homozygous susceptible F_3 lines expected, since susceptible F_2 plants (except escapes) had no seed.

* Significant at the 5 per cent level.

** Significant at the 1 per cent level.

Seven genes were associated with different chromosomes in this study. It should be pointed out that genes are not tested against linkage testers, but against whole chromosomes when nullisomics or monosomics are used. Thus, it is possible to associate with a certain chromosome genes that are more than 50 crossover units apart, in which case the conventional method would fail to reveal linkage.

The use of these aberrations simplifies considerably the genetic analysis of characters controlled by multiple genes. This was shown in the study of awning where unusual segregation occurred in families involving three different chromosomes, thus indicating that at least three gene pairs affected awning. In the study of habit of growth it was possible to associate one of the duplicate recessive genes for winter habit of growth in Hymar with a particular chromosome. In families involving chromosome IX, only the dominant allele of Chinese Spring, not associated with this chromosome, was segregating. This resulted in a modification of the duplicate gene F_2 ratio of 15 : 1, to a monohybrid 3 : 1 ratio. Had the second recessive gene been carried by one of the other 16 chromosomes tested, a similarly modified ratio would have occurred in the families of the chromosome involved. Whether or not the same results would be obtained with other duplicate recessive genes is not known.

While the Martin gene for resistance to bunt, or the gene conditioning reaction to leaf rust, was not associated with a particular chromosome, it is significant that a gene with a modifying effect on the Martin gene was associated with a particular chromosome in the study of smut reaction in the Federation 41 crosses. Whether this is the same gene, or locus, that was involved in the studies reported by Briggs (7, 8, 9), and Churchward (11), where modifiers were operating, cannot be ascertained at this time.

Cytological studies together with genetic analysis will be needed to guard against the drawing of erroneous conclusions should certain types of aberrations be encountered. Common wheat undoubtedly has many reduplicated segments of chromosomes (as do other polyploid species), and it should be recognized that univalent chromosomes might become involved in reciprocal translocations with chromosomes that are partially homologous. The resulting chromosomes would have changed genetic constitutions, and the association of a gene with a certain chromosome could be erroneous if such a gene was carried by one of the chromosomes involved in the interchange. The relative instability of univalent chromosomes results in another type of difficulty. Misdivision, especially common in univalent chromosomes (cf. Review of Literature), can result in telocentric chromosomes and isochromosomes which might seriously affect the results obtained. If, in the study of spike density, the chromosome arm carrying the recessive allele had been retained, it is doubtful if segregation in families involving chromosome XX would have differed markedly from that in families involving the other 16 chromosomes, since telocentric chromosomes in common wheat appear to be transmitted to approximately as many progeny as are normal chromosomes.

Mutant characters can be obtained in common wheat with difficulty (i.e. in low frequencies) as a result of irradiation. They can be produced with a considerably higher frequency in durum wheat, but many would undoubtedly be concealed or "covered" by duplicate genes should an effort be made to transfer such characters from the durum to common wheat. Thus, there appears to be no simple easy way in which a large number of easily classifiable, simply inherited characters could be obtained for cytogenetic study.

The sterility of nullisomics, and the high percentage of natural crossing on such plants, might well lead to difficulties in classification and erroneous conclusions, especially if natural crosses could not be distinguished phenotypically. In two phases of this study there was evidence of high percentage of natural crossing.

It seems imperative that cytologic studies should supplement the genetic studies if these deficiencies are used. Undoubtedly further studies with these deficiencies should be undertaken, since the results of this study indicate that more definite genetic and cytogenetic information can be obtained than by the conventional methods hitherto used.

SUMMARY

Seventeen monosomic (or corresponding nullisomic) lines of common wheat, var. Chinese Spring, were tested for their effect on the transmission of genes that distinguished this variety from two other (male parent) varieties, Hymar and Federation 41. Seven of the nine genes studied were associated with particular chromosomes.

1. The dominant gene for red colour of glumes in Federation 41 was associated with chromosome I.

2. Winter habit of growth in Hymar was conditioned by duplicate recessive genes, one of which was associated with chromosome IX.

3. Although Chinese Spring and Hymar are both almost awnless, awned F_2 plants were obtained. Awning in this cross was apparently conditioned by three genes. Recessive alleles of the two dominant awn-inhibiting genes of Chinese Spring were associated with chromosome VIII and X of Hymar. A dominant awn-inhibiting gene with greater effect than either of the two Chinese Spring awn inhibitors was associated with chromosome IX in Hymar. These results supplement the findings of Sears (52), and O'Mara (42).

4. Chinese Spring and Federation 41 differed by one gene pair in their reaction to leaf rust. This gene pair was not associated with any of the 17 chromosomes tested.

5. The Martin gene for resistance to bunt was not associated with a particular chromosome in either cross. In the crosses with Federation 41, a gene which modified the effectiveness of the Martin gene was associated with chromosome XVI.

6. The dense spikes of Hymar were found to be conditioned by one main dominant gene pair which was associated with chromosome XX. However, transgressive segregation indicated that at least one additional gene was modifying the degree of density or laxity of spikes.

7. A telocentric chromosome, presumably consisting of one arm of chromosome XX in both heterozygous and homozygous condition, was found in a number of F_3 lines of the Hymar crosses. It was not homologous

with the arm of chromosome XX which carried the gene for spike density, and was evidently present in the original female parent plant used in making the cross.

8. The advantages and limitations of monosomic (or nullisomic) lines, for cytogenetic studies have been discussed.

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NOTE ON THE SUSCEPTIBILITY OF QUEEN AND WORKER LARVAE OF THE HONEYBEE TO AMERICAN FOULBROOD[1]

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Worker larvae of the honeybee are most susceptible to American foulbrood during the first day of larval life after which susceptibility decreases until at about two days and five hours after hatching they can no longer be infected (1, 4, 5). This period of susceptibility corresponds roughly with the period of feeding of the larvae with royal jelly (1, 4); thereafter they are fed honey and pollen. This well recognized fact suggests that infection or perhaps spore germination may be related in some manner to the royal jelly consumed. If this is true, then queen larvae which are fed royal jelly exclusively should remain susceptible over their entire larval period or at least for an appreciably longer time than worker larvae. It is well established that queen larvae are susceptible to American foulbrood (3, 5) but no attempt has been made to determine their age of susceptibility. Studies were therefore initiated with both queen and worker larvae to obtain information on this point.

EXPERIMENTAL

Brood combs were placed in nuclei for six hours after which the combs containing eggs were either left in dequeened nuclei or removed and placed above a queen excluder in normal colonies for three days. At 6-hour intervals thereafter combs were removed and the larvae sprayed with a suspension of AFB spores so that the brood area of each comb received about one billion spores. The combs were then returned to queenless nuclei or to normal colonies above a queen excluder. A considerable number of larvae were removed from these combs by the bees; consequently, when the brood was capped, the combs were transferred to an incubator maintained at 90°-92° F. until emerging time, when each cell was examined. Diseased material was removed for microscopic examination and culturing (2). The results of two experiments, one with larvae 0–54 hours old (in dequeened nuclei) and one with larvae 48–108 hours old (kept above a queen excluder), are assembled in Table 1. It is interesting to note that many queen cells were developed in the dequeened nuclei and were thus available for comparison with worker larvae. Since many larvae had been removed from most of the combs no attempt was made to calculate the percentage of infection. It is quite obvious that both worker and queen larvae were susceptible up to 54 hours; these results agree very closely with those of Woodrow (4) with sprayed larvae.

Difficulty was experienced in the studies with queen larvae owing to extensive removal of sprayed and, to some extent, of inoculated larvae. In the first series, 12 larvae, 0–14 hours old, were grafted on each of five bars and each bar sprayed with a suspension of 100 million spores at the time intervals given in Table 2. In the second series 60 worker larvae (20–48 hours old) were grafted on six bars and left in cell-building colonies for 24 hours. They were then removed and new larvae of selected age

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TABLE 1.—SUSCEPTIBILITY OF HONEYBEE LARVAE TO AMERICAN FOULBROOD

Larval age (hours)	Number of capped cells	Number of diseased larvae
0 - 6	20 (2 queen)	2 queen
6 - 12	10 (1 drone)	1 drone, 1 worker
12 - 18	12	2 worker
12 - 24	68 (5 queen)	3 queen, 15 worker
24 - 30	1 (queen)	1 queen
30 - 36	3 (3 queen)	1 queen
36 - 42	10 (7 queen)	4 queen, 3 worker
42 - 48	36 (3 queen)	3 queen, 15 worker
48 - 54	56 (6 queen)	3 queen, 25 worker
18 - 24 (check)	45	43
48 - 54	111	6
54 - 60	334	0
60 - 66	91	0
66 - 72	43	0
72 - 78	9	0
78 - 84	54	0
84 - 90	182	0
90 - 96	141	0
96 - 102	14	0
102 - 108	191	0

TABLE 2.—SUSCEPTIBILITY OF QUEEN LARVAE OF THE HONEYBEE TO AMERICAN FOULBROOD

Larval age (hours)	Sprayed larvae	
	Capped cells remaining (out of 12)	Number diseased larvae
0 - 24	0	—
24 - 42	0	—
42 - 60	2	0
60 - 78	5	1
78 - 96	5	0
	Individually inoculated larvae	
18 - 30	0	—
48 - 60	4	3
60 - 72	10	1
72 - 84	6	0
84 - 96	10	0
96 - 108		0

(18–30 hours) were grafted. The individual larvae were inoculated with one platinum loopful (2 mm. diam.) of a heavy suspension of spore material placed on the royal jelly near the mouth parts, at given intervals (Table 2); they were then returned to the colonies. Most of the sprayed larvae, as well as some of the inoculated larvae, were removed by the bees shortly thereafter. The capped cells were therefore removed to the incubator and examined at emerging time. It will be noted that of the few larvae remaining in the sprayed set one, 60–78 hours old, was infected. Results with larvae inoculated individually were more satisfactory; three of four cells

(48–60 hours old) and one out of 10 cells (60–72 hours old) were susceptible. It would seem that queen larvae are slightly more susceptible than worker larvae which could not be infected beyond the age of 54 hours. However, considering the possible variation in age of larvae selected for infection, as observed by Woodrow (4, 5), it is questionable whether the time difference involved is significant. It is quite possible, for example, that the susceptible queen larvae in the 60–72 hour set was 60 rather than 72 hours old. It appears therefore that continuous feeding on royal jelly has no appreciable influence on susceptibility of larvae to American foulbrood. This phenomenon may be associated more intimately with physiological or other changes which the larva undergoes during its third day of growth.

SUMMARY

Studies were initiated to compare the age of susceptibility to American foulbrood of worker larvae and queen larvae of the honeybee and thereby to determine if continuous feeding on royal jelly affects susceptibility.

Worker larvae sprayed with AFB spores at 6-hour intervals from 0 to 108 hours were found to be susceptible up to but not beyond 54 hours.

Queen larvae, individually inoculated with AFB spores, were susceptible between the age limits of 48–60 hours; one out of 10 larvae 60–72 hours old was also infected.

Considering the possible variation in age of larvae selected for experimentation it was concluded that there was no significant difference between the ages of susceptibility of queen and worker larvae, and therefore that continuous feeding on royal jelly does not appear to affect susceptibility to AFB.

ACKNOWLEDGMENT

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CYTOGENETIC STUDIES OF AN INVERSION IN MAIZE[1]

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In higher plants, pollen sterility frequently has been found to be the result of abnormal chromosome behaviour or of structural changes in the chromosomes such as translocations, inversions and deficiencies. The degree of sterility may vary from low to high and may or may not be associated with the same degree of ovule abortion, depending on the nature of the abnormality and the species. These changes may occur naturally, or may be induced by X-rays or other irradiations. This is a report of studies in corn, *Zea mays*, of an X-ray produced stock which shows a low degree of pollen abortion without corresponding ovule abortion.

In maize, pollen sterility due to heterozygous reciprocal chromosomal translocations is accompanied by approximately the same degree of ovule abortion, usually about 50 per cent, Brink and Burnham (2), Burnham (5), although some with only about 25 per cent have been reported, Burnham (4) Clarke and Anderson (8), Clark (7). The former have at diakinesis a ring of four chromosomes plus eight pairs most frequently; while the latter have a chain of four chromosomes plus eight pairs as the usual configuration as the result of one break being in the satellite. In the latter it was suggested that the deficiency for a piece of the satellite in one of the classes which normally aborts fails to cause abortion, thus giving 25 per cent of sterility rather than 50 per cent. This class was shown to be functional in female gametes, Burnham (4).

Pollen sterility in maize, not accompanied by an equal amount of ovule abortion, has been reported rather infrequently. A gene for pollen abortion *pa* has been reported in an inbred line by Burnham (6). Plants heterozygous for this gene produced semi-sterile pollen, but normal ears. As this gene is lethal to pollen carrying it, but not to ovules, *pa* is transmitted mainly, possibly entirely, through the ovules. Genetic data placed *pa* in chromosome 1 but no cytological abnormality could be located although it was pointed out that a very small deficiency might not have been recognizable.

Clark (7), in studying the causes of partial sterility in inbred lines and hybrids of corn, investigated pollen and ovule semi-lethals in which semi-sterility is a result of gene action rather than of chromosome aberrations. One of these types produced pollen which was 30 per cent aborted but had normally filled ears. A cytological examination of the chromosomes showed that they were all apparently normal. She suggested that there could have been a chromosomal aberration present, so small that it was not detected in the cytological examinations.

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In the same study she found one inversion which caused low pollen and ovule sterility. This inversion was of the pericentric type which includes the centromere. A second inversion of the paracentric type which does not include the centromere was reported but the amount of male and female sterility was not given.

McClintock [unpublished observations cited by Smith (15)] has observed that inversions (and other aberrations which give rise to bridge configurations at meiosis) do not produce female sterility proportionate to the number of anaphase cells with bridges. In her reports (13, 14), she has not given the pollen and ovule abortion in plants heterozygous for the inversions.

Smith (15) reported an inversion in a strain of barley. One plant, at the first meiotic division of the pollen mother cells, had 24 per cent of the cells with either a bridge or both a bridge and a fragment, while a second had 14 per cent. Female fertility was not much reduced in the first plant, there being a seed in all but one of 45 primary florets.

Sturtevant and Beadle (18), in a study of inversion heterozygotes in *Drosophila melanogaster*, suggested a possible explanation for the absence of inviable zygotes resulting from single crossovers in the inverted segment. Meiosis in the oocytes results essentially in a linear quartet of nuclei. A single crossover in the inverted section during the first meiotic division results in a dicentric chromatid which forms an anaphase bridge. At the second division this bridge is assumed to hold the crossover chromatids together and only the non-crossover ones are free to move into the terminal nuclei. Since it is always one of these terminal nuclei that functions as the egg nucleus, it is evident that only a non-crossover chromatid can be recovered, and no egg sterility would be observed. Since there is a linear quartet of megaspores in maize, a similar result might be expected.

DESCRIPTION OF STOCK AND RESULTS OF FIRST STUDIES

The partially sterile line of maize used in this study came from a culture grown from seed* produced by the application of X-rayed pollen on a normal plant. It was one of a group showing partial pollen sterility and 10 pairs of chromosomes at diakinesis which had been selected in 1940 during a search for new translocations involving chromosome 6. It was designated as sterile line number 6071.

The original plant from which this culture came had an estimated 15 per cent of its pollen grains wholly or partially devoid of starch while the ear appeared normally filled. The breeding behaviour in later generations was checked in progeny from the selfing of partially sterile plants and also from crosses with normals using them as the male and as the female parents. In the progeny derived from selfing, about 50 per cent of the plants had partially sterile pollen. In the progeny of crosses with standard normals using a partially sterile plant as the female parent, about 35 per cent of the plants from one cross and about 50 per cent from a second were classified as partially sterile. When crossed as the male parent, about 50 per cent of the progeny in two crosses and about 30 per cent in a third cross were "low sterile" plants. As before, examination of the ears indicated that the abnormality did not cause ovule abortion. Since the sterility was transmitted through both male and female, plants with normal pollen from a cross of low sterile × low sterile were self-pollinated and crossed with a standard normal line as a test to determine if a stock homozygous for the abnormality could be established. Cytological examination of microsporocytes of partially sterile plants revealed 10 pairs of chromosomes, all apparently normal at diakinesis. Also the spore quartets were normal as to nucleolar appearance (one per spore).

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*Furnished by L. J. Stadler.

Partially sterile plants were crossed with genetic markers in the various linkage groups and the partially sterile F_1 plants were backcrossed to the normal recessive stock. At this point the first writer took over the study, conducted the linkage tests, the pachytene analysis of the abnormality and further pollen sterility studies.

Pollen examination of the test crosses to establish a stock homozygous for the abnormality showed six crosses with all normal plants and five crosses with all low sterile plants. Consequently, about 50 per cent of the normal plants from low sterile 6071 × low sterile 6071 were actually homozygous for whatever produced the low sterility character. Pollen counts to determine the amount of abortion were made on these same plants and are in Table 1. In general, there was a clear-cut division between sterile and normal grains, although a few smaller, almost completely filled, grains were present in each sample. In these progenies, the variation in sterility between plants within a cross was rather small, and was not much greater between progenies. A certain amount of variation is expected due to environment and to sampling from the anther. Progeny A5730 was studied for the variation between florets within a single plant. This variation was found to be quite small, ranging only from 16.3 per cent to 19.4 per cent. For all the plants, pollen abortion varied from 12 to 22 per cent, the average being 16.5.

The proportions of low sterile plants in the various progenies of crosses between low sterile and normal plants, omitting those where there may have been disturbances due to linkage of mutant characters with the

TABLE 1.—POLLEN COUNTS OF F_1 PROGENY FROM CROSSES OF STANDARD NORMAL LINES × LINES HOMOZYGOUS FOR THE CAUSE OF THE LOW POLLEN STERILITY (No. 6071)

Progeny	Number of normal grains	Number of aborted grains	Total	Percentage aborted	Average
A5730-1	5480	1192	6672	17.9	
-2	1977	468	2445	19.1	18.5
-3	753	208	961	21.6	
A5731-1	944	142	1086	13.1	
-2	816	111	927	12.0	12.6
A5734-1	1178	176	1354	13.0	
-2	1055	150	1205	12.4	12.7
A5735-1	955	197	1152	17.1	
-2	1398	284	1682	16.9	15.9
-3	873	127	1000	12.7	
A5736-1	901	173	1074	16.1	
-2	796	152	948	16.0	16.1
Totals	17,126	3380	20,506		16.5

TABLE 2.—DATA ON BRIDGES AND FRAGMENTS AT THE FIRST MEIOTIC DIVISION IN POLLEN MOTHER CELLS OF LOW STERILE F_1 PLANTS FROM A CROSS OF STANDARD NORMAL × HOMOZYGOUS LINES

Total sporocytes	Bridge		No bridge		Per cent irregular cells
	Fragment	No fragment	Fragment	No fragment	
			Early anaphase I		
53	24	15	0	14	73.6
			Late anaphase I		
33	6	5	2	20	39.4
			Telophase I		
216	14	5	34	163	24.5
Total 302	44	25	36	197	26.5

sterility, varied from 25.7 to 70.4 per cent for progenies of individual ears, many of the deviations from 50 per cent being highly significant. The percentage on the total, however, was 49.3. All of the progenies but one, which had 65.3 per cent of low sterile plants, resulted from crosses using a partially sterile plant as the female parent.

CYTOLOGICAL RESULTS

Although the cytological results were not obtained until after the first linkage tests were completed, they will be presented first since they indicate the kind of change which had occurred. All cytological work was done at University Farm, St. Paul. For this study plants homozygous for the change producing sterility 6071, plants heterozygous for it, and standard normals were grown at University Farm, St. Paul, in the greenhouse. The sporocytes were killed and fixed in three parts 95 per cent ethyl alcohol and one part glacial acetic acid, and later transferred to 70 per cent ethyl alcohol for storage in the refrigerator. The aceto-carmine smear technique was used for cytological examinations. Camera lucida drawings, magnified 4500 × on the paper, were made of a number of cells exhibiting chromosome abnormalities. Photographs of three of these drawings, reduced 20 per cent, are presented here.

The fact that the sterility was present in the male and not in the female, but was transmitted through both, suggested that an inversion might be present in one of the chromosomes. Consequently, emphasis was placed on a study of the pachytene and anaphase stages of meiosis. A few preparations of diakinesis and metaphase I were examined but, in accord with the earlier observations, no irregularities were seen.

Anaphase and telophase stages of the first meiotic division showed a number of cells each of which contained a bridge and a fragment. Counts made on these irregularities are given in Table 2. They are based on three slides from two plants of progeny A5735, all of which should have been similar. The presence of the bridges and fragments indicates the presence of an inversion which does not include the centromere, i.e. the paracentric

TABLE 3.—DATA ON FRAGMENTS AT THE SECOND MEIOTIC DIVISION IN POLLEN MOTHER CELLS OF LOW STERILE F_1 PLANTS FROM A CROSS OF STANDARD NORMAL × HOMOZYGOUS LINES

Total cells	Fragment	No fragment	Per cent irregular cells
		Metaphase II	
167	16	151	9.6
		Anaphase II	
24	5	19	20.8
		Telophase II	
134	16	118	11.9
Total 325	37	288	11.4

type. The bridge plus fragment configuration at anaphase I is the result of a single crossover or of a 3-strand double crossover within such an inversion. Two-strand double crossovers do not result in bridges and fragments since the chromatids are normal. On the other hand, a 4-strand double crossover is expected to give rise to two bridges plus two fragments at anaphase I. No double bridges were identified in heterozygous 6071, suggesting that very little double crossing over occurred within the inverted segment.

The chromatids having a single crossover, whether from an actual single crossover, or from 3- or 4-strand doubles, carry chromosomal duplications and deficiencies and in seed plants are expected to result in aborted spores. The degree of pollen sterility should be related to the frequency of crossing over. Sibs of these plants grown in the field had 15.9 per cent pollen abortion. If, on the average, only two cells of a quartet in which crossing over has taken place develop into normal pollen grains, the number of cells expected to show abnormalities at anaphase I would be 2 × 15.9 or 31.8 per cent. A much higher proportion of abnormalities than this was observed at anaphase I. The number observed at telophase was considerably less. The number of cells on which the anaphase observation is based was very small; also certain late disjunctions with no fragments may have been erroneously recorded as bridges. A closer relation might have been obtained if pollen counts had been obtained on the plants examined cytologically (grown in the greenhouse), but unfortunately the entire tassel was taken for sporocytes. The camera lucida drawing shown in Figure 1 is typical of cells which showed anaphase bridges and fragments during the first division. It will be noted that the size of the fragment is rather large as compared with the size of the chromosomes. In many cases, the bridge had been broken before reaching telophase I.

Counts made on abnormalities in division II are shown in Table 3. It is readily seen that the number of cells with fragments at division II is considerably less than the number with bridges and fragments in division I. This may be due to the association of the fragment with a normal chromatid in the bridge figure as observed by McClintock (14). No bridges were seen in any of the second division figures. As shown by McClintock (14), these occur when certain strands cross over between the centromere and the

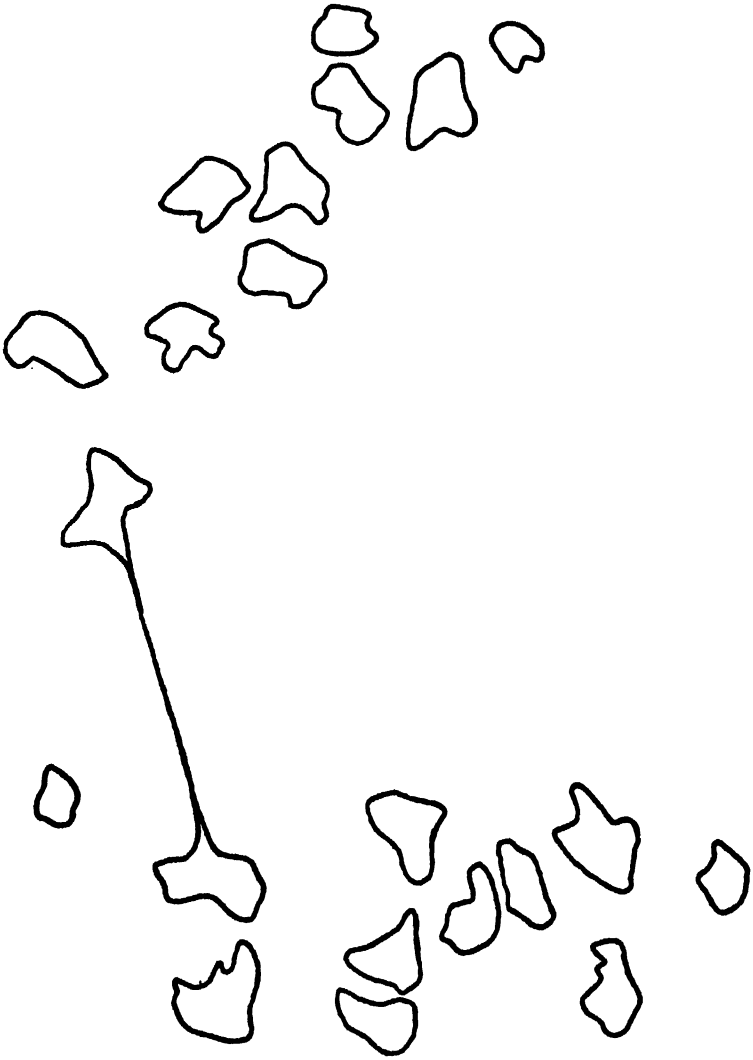
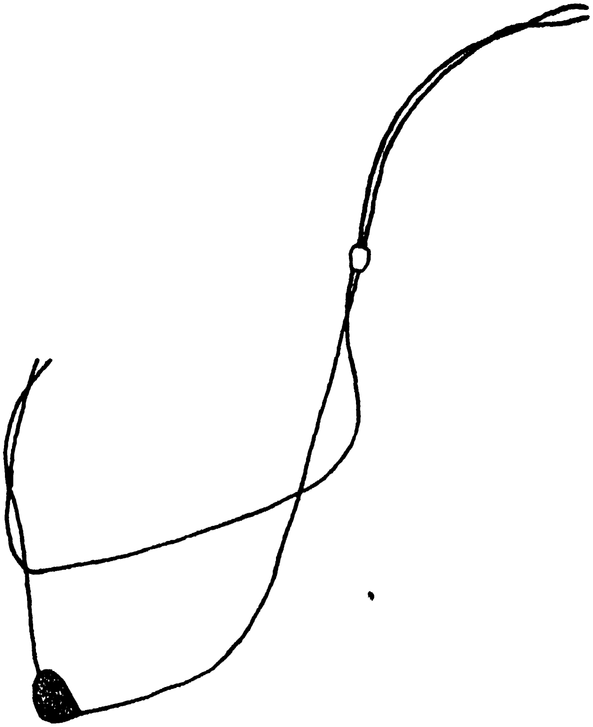


FIGURE 1. Camera lucida drawing (X 3600) of anaphase I in a plant (heterozygous for an inversion in the long arm of chromosome 2.) Note the single bridge and free fragment.

inversion loop simultaneously with certain single crossovers within the loop. Since no anaphase II bridges were seen, such simultaneous crossovers must have occurred infrequently if at all.

A large number of pachytene cells was examined for the pairing behaviour of the chromosomes. Very few cells were obtained with the proper staining of chromosomes to show individual members of a pair. During these observations, chromosomes 1, 3, 6, 7, 8, 9, and 10 were identified, and they appeared to be normal in all respects. Six configurations were seen which were similar to the inversion figures presented by McClintock (13, 14). Measurements taken on the chromosome concerned



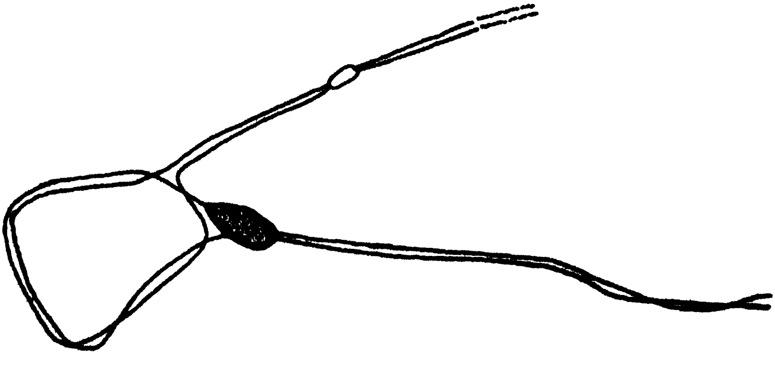


FIGURE 2 *(Top)*. Camera lucida drawing (X 3600) showing asynapsis of a normal chromosome and its homologue possessing the inversion. The clear oblong area is the centromere.

FIGURE 3 *(Bottom)*. Camera lucida drawing (X 3600) showing synapsis at pachytene of a normal chromosome and its homologue possessing the inversion.

and a comparison with other chromosomes indicated it was chromosome 2. In all cases but one, the region involved was in the long arm between the subterminal pycnotic body and the centromere (Figure 3). This one exception, shown in Figure 2, probably exhibits asynapsis of homologous parts at the distal side of the pycnotic body. Measurements of the loop and the long arm showed that about 50 per cent of the total length of that arm was involved in the inversion. Unfortunately, in none of the cells were the chromomeres stained clearly so that all the single strands at the cross were distinct enough to be seen in a photograph. However, the loop could not be mistaken because of the characteristic shape and position of the knob, the size of the loop and the location of the centromere.

The lengths and relative sizes of the products of crossing over may be calculated on the basis of the breakage point locations. The length of the acentric fragment is equal to the length of the pachytene loop plus twice the length of the portion of the long arm distal to the loop. The length of the dicentric chromatid, based on a standard cytological map where number 10 is 100, is 242.5, whereas the length of the acentric chromatid is 149.5. Associated with each centromere of the dicentric is a normal chromatid, the length of which is 196. Therefore, placed on a relative pachytene length basis, the normal chromatid plus half the dicentric, or 196 + 242.5/2 = 317.25, will be at each pole and should be 2.1 times the acentric chromatid, or fragment, which is 149.5. This is roughly what it appears to be in Figure 1.

A few slides were prepared from anthers of the homozygous 6071 plants to determine whether or not the positions of the breaks in the chromosome could be located by means of the chromomere pattern. Chromosome 2 in the X-rayed stock carried the pycnotic body and therefore this region is expected to be with the inversion. Although chromosome 2 was identified with certainty twice in the large number of cells examined, no distinct changes in chromomere pattern indicating a breakage point were seen. It was noted, however, that the relative position of the pycnotic region in chromosome 2 appeared the same for homozygous 6071 as for standard normal plants. On this basis and also from the location of the pycnotic region in relation to the loop in the heterozygote, it seems reasonable to conclude that one of the breaks is near this dark staining body, but that it is not included in the inversion. Measurements of the pachytene figures place one break at approximately 0.1 of the distance from the centromere to the end of the long arm, the other at about 0.6.

LINKAGE TESTS

The field tests for linkage were made during the summer of 1946 at the Dominion Experimental Station, Morden, Manitoba. Supplementary data for linkage group 2 were obtained from backcross progeny grown at the University Farm, St. Paul, Minnesota, during the summer of 1947. The crosses involved the tester characters for the ten linkage groups as shown in Table 4. The positions of the genes and the probable positions of the centromere (S.F.) in the linkage map are also shown, based on Anderson and Randolph (1). The end of the map nearest the end of the short arm of the chromosome is at the left. Only in group 2 were both arms adequately tested. In group 5, the crosses made to give segregation for *a_2* and *bm* showed no segregation for sterility. Usually two or three backcrossed ears were tested for each group. Enough seed was planted to furnish a planned minimum of 400 plants for each linkage group. Unfortunately, adverse weather conditions after planting in May caused very poor germination in certain classes, particularly sugary and shrunken

TABLE 4.—LIST OF GENES USED AS TESTERS FOR EACH LINKAGE GROUP, TOGETHER WITH THEIR MAP LOCATIONS AND THAT OF THE CENTROMERE (S.F.), THE LATTER BASED ON ANDERSON AND RANDOLPH (1)

Linkage group	Name of gene used and symbol, and respective chromosome maps*
1	bm_2 brown midrib-2, *br*—brachytic, *f* fine stripe-1, *P*—pericarp and cob colour. *P* S.F. *br* *f* bm_2 28 86 91 166
2	*B* plant colour booster, gl_2—glossy seedling-2, *lg*—liguleless leaf-1, v_4—virescent seedling-4, *Ch* chocolate pericarp. *lg* gl_2 *B* S.F. v_4 *Ch* 11 30 49 82 124
3	*a* aleurone, plant, and pericarp colour-1, *na* nana-1, ts_4—tassel seed-4. S.F. ts_4 *na* *a* 55 83 111
4	gl_3 glossy seedling-3, *su*—sugary endosperm-1, *Tu*—tunicate ear. *su* S.F. *Tu* gl_3 71 107 118
5	A_2—aleurone colour, *pr*—red aleurone, v_2—virescent seedling-2. a_2 S.F. *pr* v_2 0 31 72
6	*Pl* purple plant colour, su_2—sugary endosperm-2**, *Y*—yellow endosperm. S.F. *Y* *Pl* su_2 13 41 49
7	*gl*—glossy seedling-1, *ra* ramosa ear-1, v_5—virescent seedling-5. v_5 S.F. *ra* *gl* 24 32 36
8	*j* japonica-1, ms_8 male sterile-8. S.F. ms_8 *j* 14 28
9	*C*—aleurone colour, *sh*—shrunken endosperm, *wx*—waxy endosperm. *C* *sh* *wx* S.F. 21 24 54
10	*g*—golden, *R*—coloured aleurone and plant. S.F. *g* *R* 43 57

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* Hayes and Immer (11), Emerson *et al.* (10).

** The position of su_2 is not certain; Burnham (unpublished data) reported it to be 7.7 units to the right of *Pl*, while Horovitz *et al.* (12) reported it to be 7.4 to the left of *Pl*.

TABLE 5.—DATA ON THE SEGREGATION OF PLANTS WITH NORMAL AND WITH PARTIALLY STERILE POLLEN IN RELATION TO GENES IN THE TEN LINKAGE GROUPS, TOGETHER WITH THE P VALUES FOR X^2 TESTS FOR INDEPENDENCE—BACKCROSS DATA

Linkage group	Genes tested		Normal pollen		Partially sterile pollen		Total plants	Number of progenies	X^2 values	P values
	X	x	X	x[2]	X[2]	x				
1	Bm_2	$-bm_2$	115	92	73	57	337	4	0.01	0.90
	Br	$-br$	112	93	67	63	335	4	0.31	0.50
	F_1	$-f_1$	117	89	74	55	335	4	0.01	0.90
	P	$-p$	88	82	45	48	263	4	0.27	0.50
2	B	$-b$	52	89	110	53	304	3	28.45	0.001**
	Ch	$-ch$	41	32	39	54	166	1	3.32	0.06
	Gl_2	$-gl_2$	60	82	90	72	304	3	3.36	0.02–0.05*
	Lg_1	$-lg_1$	66	76	80	82	304	3	0.26	0.05
	V_4	$-v_4$	24	118	139	23	304	3	143.98	0.001**
3	A_1	$-a_1$	40	108	41	92	281	6	0.49	0.30
	Na_1	$-na_1$	44	12	37	23	116	2	3.93	0.02–0.05*
	Ts_4	$-ts_4$	64	35	51	50	200	3	4.10	0.02–0.05*
4	Gl_3	$-gl_3$	90	45	98	33	266	3	2.13	0.10
	Tu	$-tu$	31	52	12	23	118	2	0.10	0.70
	Su_1	$-su_1$	70	13	26	9	118	2	1.64	0.20
5	Pr	$-pr$	122	78	81	48	329	7	0.11	0.70
	V_2	$-v_2$	67	35	83	57	242	1	1.03	0.30
6	Pl	$-pl$	6	1	25	8	40	1	0.01	0.95
	Su_2	$-su_2$	86	93	80	73	332	2	0.59	0.30
7	V_5	$-v_5$	53	39	112	81	285	3	0.01	0.90
	Ra	$-ra$	62	27	136	54	279	3	0.11	0.70
	Gl_1	$-gl_1$	63	29	136	57	285	3	0.12	0.70
8	Ms_4	$-ms_8$	27	—	43	—	70	1	3.66	0.05–0.10
	J_1	$-j_1$	32	10	46	9	97	2	0.84	0.30
9	C	$-c$	49	18	43	17	127	2	0.03	0.80
	Sh	$-sh$	84	34	70	37	225	3	0.86	0.30
	Wx	$-wx$	104	76	73	52	305	4	0.01	0.90
10	R	$-r$	26	6	67	51	150	2	5.40	0.02[1]–0.05[1]*
	Gl	$-gl$	20	6	50	40	116	1	3.01	0.05–0.10[1]
?	+	– –								
	aleurone[3]		94	112	62	67	335	4	0.19	0.50
		“	70	72	84	77	303	3	0.07	0.70
		“	65	83	65	66	279	6	0.91	0.30
		“	59	42	58	60	219	2	1.88	0.10
		“	14	28	23	32	97	2	0.75	0.30
		“	49	18	43	17	127	2	0.03	0.80
?	Y	$-y$[4]	73	73	32	43	221	3	1.07	0.30
		“	33	59	70	123	285	3	0.00	0.90

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[1] Continuity correction applied.

[2] Parental classes except for *P*, *Ch* and *Tu*.

[3] + and – = coloured vs. colourless aleurone.

[4] Probably $Y - y$ in chromosome 6 but not certain.

* Significant.

** Highly significant.

endosperm in groups 4 and 9, respectively. Even with supplementary seedings made the second week in June, the numbers still did not reach the number planned, but are believed sufficient in most cases to detect the presence of anything but very loose linkage.

Crosses segregating for seedling characters were classified early after emergence; the recessives which would not be recognizable later were staked and rechecked several times. After complete emergence, individual plant notes were taken. Thereafter, the segregation for plant characters was recorded at the time when they became most apparent. In most cases, the tester characters could be classified in advance of maturity so that the late-seeded material was as useful as that seeded earlier.

A pollen sample from each plant was examined to determine whether it was normal or partially sterile. For this purpose, a small piece of the tassel ready to shed pollen was collected, an anther teased out in iodine-potassium iodide solution on a slide and examined with a binocular microscope. Any sample with over 5 per cent of empty or only partly filled grains was classified as partially sterile. After first counting and estimating a few samples to ascertain the characteristic appearance, the classifications were made by estimation.

After all field characters were recorded and the pollen samples classified, the data were assembled and grouped to test for linkage using the chi-square (X^2) test for independence, by use of the formula

$$X^2 = \frac{(ad - bc)^2 N}{(a + b)(a + c)(b + d)(c + d)}$$ with one degree of freedom.

The progenies in the group showing linkage with pollen sterility were tested for heterogeneity by the X^2 method. P values smaller than 0.05 are considered significant and smaller than 0.01 highly significant. Where association was indicated recombination values were calculated.

A summary of the data for the four phenotypes involving pollen sterility and each qualitative genetic character is given in Table 5. A similar summary of data in which the particular genes responsible for the genetic characters are not definitely known, aleurone and endosperm colours, is in the latter part of this table. For all except *Tu* in group 4, *P* in group 1, and *Ch* in group 2, partial sterility entered the cross with the dominant character. Hence, with these exceptions, X sterile and x normal are the parental classes.

The P values given in Table 5 indicate that, with the exception of groups 2, 3, and 10, there is no association between pollen sterility and the genes tested. In linkage group 1 (*bm*, *br*, *f*, *P*), the total number of plants used to test the *P* vs *p* genes was less than for the other genes because of a heavy smut epidemic on ears. In group 4 (*su*, *gl*, *Tu*) very poor germination of the sugary endosperm seed in this group decreased the stand appreciably. The number of virescent seedlings present in group 5 (*pr*, v_2) was fewer than expected from a normal backcross because many of them died shortly after emergence. The classification into starchy and sugary endosperm in group 6 was good but, due to the inexperience of the writer, there may have been some misclassification of the plants segregating for *Pl* vs. *pl*. They normally show up in *b* plants as purple vs. reddish anthers, and in *B* plants as purple vs. sun red plants. If the r^g allele is present along with *b*, the two classes cannot be distinguished. A relatively small number of plants was available for the test in group 8 (j, ms_8) because of poor germination. Also, since the male sterile plants produced no pollen, segregation for this character versus pollen sterility could be studied only by classifying the non-male sterile plants. There is a possibility that the yellow vs. white endosperm colour segregation was due to the factor pair *Yy* located in chromosome 6. If so, this gives further support for the absence of any change in chromosome 6 as the cause of pollen sterility.

As pointed out above, long regions of many of the chromosomes were not covered in the linkage tests, making it impossible to state definitely from these tests that a certain chromosome was not involved in the production of sterility.

The tests which appear to indicate linkage will now be discussed.

Tests for Linkage Group 3—a, ts_4, na

The genes used (*a*, ts_4, *na*) are all located in the long arm and entered the cross together. In addition to low viability of seed, in several cases, *na* ts_4 plants did not shed any pollen. As none of the tassel seed plants shed pollen readily at any time, at least not until late, some tassel branches may have been collected too early for a satisfactory classification for sterility. The data show a much higher proportion of these was classed as partially sterile than of the ts_4 or *Na* classes. The P value of 0.02-0.05 for *na* and for ts_4 appears to indicate linkage between sterility and each of these characters; however, a check of the data shows that the association is not in the direction expected, i.e. it is not between the characters which entered the cross together. This is shown also by a calculation of the recombination value which for ts_4 is 57.0 ± 3.5 and for *na* is 57.8 ± 4.6, i.e. above 50 per cent in both cases. Hence there is no good evidence for association of partial sterility with this linkage group.

With *r* and *g* in group 10, there were few plants because one cross produced all plants with semi-sterile pollen and was omitted and a second did not segregate for golden plants. Since the two progenies which did segregate for pollen sterility produced more plants of this class than expected from a backcross, and the degree of sterility of many of these was considerably higher than in the other groups, it is probable that some other abnormality was present also; the test was unsatisfactory. Even assuming this is not true, here, again, the parental classes are fewer than the new combinations and the P value of 0.02-0.05 cannot be interpreted to mean association.

Tests with Linkage Group 2—B, gl_2, lg, v_4, Ch

The genes used in this study were well distributed along the entire length of the chromosome as shown in Table 4. The segregation for chocolate (*Ch*) and colourless pericarp was not obtained in 1946 due to late maturity but was secured in supplementary data collected in 1947.

The summary of results in Table 5 shows P values which indicate linkage of the pollen sterility with each of the genes *B*, v_4, and gl_2, but not for *lg* or *Ch* in the same chromosome. Since the P values from X^2 for

TABLE 6.—SUMMARY OF TWO-POINT LINKAGE DATA FOR PARTIAL STERILITY AND GENES IN CHROMOSOME 2, BACKCROSS DATA. THE 304 TOTALS ARE 1946 DATA; THE 166 TOTALS ARE 1947

Genes tested, group 2	Normal pollen		Partially sterile pollen		Total plants	Recombination values ± S.E.
	X	x	X	x		
B - *b*	52	89	110	53	304	34.5 ± 2.7
Ch - *ch*	41	32	39	54	166	42.8 ± 3.8
Gl_2 - gl_2	60 29	82 44	90 42	72 51	304 166	43.4 ± 2.8 48.2 ± 3.9
Total	89	126	132	123	470	45.1 ± 2.3
Lg - *lg*	66 37	76 36	80 58	82 35	304 166	48.7 ± 2.9 43.4 ± 3.8
Total	103	112	138	117	470	46.8 ± 2.3
V_4 - v_4	24 12	118 61	139 85	23 8	304 166	15.5 ± 2.1 12.0 ± 2.5
Total	36	179	224	31	470	14.3 ± 1.6

heterogeneity for the associations with sterility are higher than the minimum level for significance in all (P = 0.1 to 0.2 for gl_2, P = 0.05 − 0.1 for *B*, P = 0.3 − 0.5 for v_4), the variations between the different progenies is no greater than expected on the basis of random sampling. These data summarized as 2-point tests are in Table 6. The recombination values between partial sterility and each character are: 15.5 ± 2.1 with virescent seedling — 4 (v_4), 34.5 ± 2.7 with plant colour booster (*B*), and 43.4 ± 2.8 with glossy seedling — 2 (gl_2). For the v_4 test, all three progenies show a highly significant and consistent deviation from 50 per cent. For *B* the deviation is significant for all but one progeny, and it is significantly less than 50 per cent on the total population. With gl_2, in only one of the three crosses is there a significant deviation from the 50 per cent of recombination. The deviation is slightly larger than 2 × S.E. and therefore significant on the total population.

The data from three crosses are presented in Table 7 as a 5-point linkage test. These data which show the numbers in the different cross-over classes indicate that the gene order is *lg* — gl_2 — v_4 — sterility. This means that the sterility character is located in the long arm distal to the location of v_4. The coincidence values have high standard errors and do not deviate significantly from 1.00. Larger populations are needed before drawing any conclusions with regard to interference across the centromere.

Additional data for linkage group 2 were obtained from a segregating population grown in 1947 at University Farm in St. Paul, Minnesota. Unfortunately, the plants were not classified for the colour booster character (*B*-*b*). The segregation for chocolate and colourless pericarp (*Ch*-*ch*), as well as for the other characters included previously in this group, was studied. Considerable difficulty was experienced in classifying a number

TABLE 7.—FIVE-POINT LINKAGE TEST, GROUP 2 IN WHICH THE F_1 GENOTYPE, $\frac{+\ +\ B\ +\ \text{sterility}}{lg\ gl_2\ +\ v_4\ \ +}$, WAS BACKCROSSED TO $lg\ gl_2\ +\ v_4\ +$, 1946 DATA

Parental combinations	Recombinations					
	Region 1	Region 2	Region 3	Region 4	Regions 1 and 2	Regions 1 and 3
57 52 109 35.9 per cent	13 19 32 10.5 per cent	18 21 39 12.8 per cent	23 27 50 16.5 per cent	8 14 22 7.2 per cent	3 1 4 1.3 per cent	4 8 12 4.0 per cent
	Regions 1 and 4	Regions 2 and 3	Regions 2 and 4	Regions 3 and 4	Regions 1, 2, 3	Regions 1, 2, 4
	0 3 3 1.0 per cent	5 4 9 3.0 per cent	2 3 5 1.6 per cent	7 6 13 4.3 per cent	2 0 2 0.7 per cent	2 0 2 0.7 per cent
	Regions 1, 3, 4	Regions 2, 3, 4	Regions 1, 2, 3, 4	Total = 304 Recombination percentages: $lg\ gl_2$ – 18.4, $gl_2\ B$ – 20.7, $B\ v_4$ – 20.0, v_4 sterility – 15.5		
	— — —	0 1 1 0.3 per cent	1 0 1 0.3 per cent			

Coincidence—Regions 1 and 2 c = 0.78 ± 0.22
Regions 2 and 3 c = 0.71 ± 0.16
Regions 3 and 4 c = 1.10 ± 0.22

TABLE 8.—FIVE-POINT LINKAGE TEST, GROUP 2 IN WHICH THE F_1 GENOTYPE $\frac{+\quad +\quad +\ \text{sterility}\ +}{lg\quad gl\quad v_4\quad +\quad Ch}$ WAS BACKCROSSED TO $lg\ gl\ v_4 + +$, 1947 DATA

Parental combinations	Recombinations					
	Region 1	Region 2	Region 3	Region 4	Regions 1 and 2	Regions 1 and 3
20 19 39 23.4 per cent	5 2 7 4.2 per cent	10 14 24 14.5 per cent	3 3 6 3.6 per cent	14 10 24 14.5 per cent	13 3 16 9.6 per cent	1 0 1 0.6 per cent
	Regions 1 and 4	Regions 2 and 3	Regions 2 and 4	Regions 3 and 4	Regions 1, 2, 3	Regions 1, 2, 4
	4 3 7 4.2 per cent	0 1 1 0.6 per cent	10 10 20 12.1 per cent	1 2 3 1.8 per cent	0 1 1 0.6 per cent	8 1 9 5.4 per cent
	Regions 1, 3, 4	Regions 2, 3, 4	Regions 1, 2, 3, 4	Total = 166 Recombination percentages: $lg\ gl_2$ – 27.7, $gl_2\ v_4$ – 47.0, v_4 sterility – 12.0, sterility Ch – 42.8		
	0 1 1 0.6 per cent	1 2 3 1.8 per cent	3 1 4 2.4 per cent			

Coincidence—Regions 1 and 2 c = 1.39 ± 0.14
Regions 2 and 3 c = 0.96 ± 0.22
Regions 3 and 4 c = 1.29 ± 0.25

of the pollen samples, in spite of their having been collected and preserved in alcohol. The 5-point data are summarized in Table 8. The 2-point data for the four phenotypes involving pollen fertility and each genetic marker are included in Table 6. Inspection of these data in Table 6 reveals no marked deviations which might result if errors in pollen sterility classification were numerous and in one direction. Here also the order is $lg - gl_2 - v_4 -$ sterility. The recombination between *Ch* and sterility is 42.8 ± 3.8 the deviation from 50 per cent approaching significance, being only slightly less than 2 × S.E. The recombination value between *Ch* and v_4 is 41.6 ± 3.8 which is practically the same as that shown in normal stocks (Table 4). The location of *Ch* is discussed later.

DISCUSSION

In this study there is no evidence for any reduction in genetic crossing over in plants heterozygous for inversion 6071. The recombination value of 29 per cent between *B* and v_4, which is 8 per cent higher than that reported by Emerson *et al.* (10), may or may not have been due to the use of different stocks. The recombination value between v_4 and *Ch* is 41.6 per cent which is practically the same as that for a normal stock, Burnham—unpublished data in Emerson *et al.* (10).

Heterozygous inversions in *Drosophila* reduce the frequency of observed genetic crossing over within the inverted segment and in adjacent regions. Experiments by Sturtevant and Beadle (18) show that they may also reduce cytological crossing over but very much less than the amount indicated by the reduction in genetic crossing over. The explanation is that crossing over occurs in the inverted segment, but most of the crossover chromatids are not recovered. In these experiments, enough crossing over occurs to produce about 16 per cent of pollen abortion, yet genetic crossing over was not measurably reduced.

If *Ch* is in the inverted segment, then 2-strand double crossovers within the pachytene loop, one on each side of this gene, would be required to give recovered recombinations between partial sterility and chocolate pericarp. Partial pollen sterility and *Ch* show 42.8 per cent recombination in this study. If 2-strand doubles occur within the loop, then 4-strand doubles should occur also, and at approximately the same frequency. Four-strand double crossovers within the inversion result in double bridges and two fragments at anaphase I. Since no such figures were seen in the cytological analysis, it appears that double crossing over may be rare in this inversion. Therefore, it seems reasonable to conclude that *Ch* is situated outside the inverted segment and on the side opposite to v_4, i.e. nearer the end of the long arm. It may be so near the end of the chromosome that it shows very little difference in recombination with v_4 or the sterility resulting from the inversion; or is it possible that the acentric fragment is able to persist into the next generation by passing along with a non-crossover chromatid by the mechanism described by McClintock (14)? This might account for the observed rather free recombination of *Ch* with v_4 and with partial sterility. Genetic studies including genes in the inversion and more genes in the distal segment between *Ch* and the outer break are needed. A cytological study of the crossovers should be made also.

The apparent absence of female sterility in plants heterozygous for this inversion (6071) is an interesting feature. This has been observed before by Smith in barley and by McClintock (unpublished) in maize. Sturtevant and Beadle's explanation (18) for the absence of inviable zygotes as a result of crossovers in an inversion in *Drosophila* is that the anaphase I bridge holds the abnormal chromatids to the centre of the dyad during the second meiotic division, leaving the normal chomatids free to move to the end cells. This may explain the lack of ovule sterility in maize. It is known that there is a linear quartet of megaspores in maize, the chalazal cell of which develops into the female gametophyte. In this study, it was observed that the bridge was broken in many figures before telophase I, and beyond recognition before completion of the cell wall, apparently leaving the chromatids free to proceed to either cell during division II. It is difficult to see how the explanation proposed for *Drosophila* operates here unless the bridge persists longer than this during meiosis in the megasporocyte, or the second division proceeds more rapidly without going into as much of an interphase. McClintock (14) presented evidence of at least part of the anaphase I bridges persisting late in meiosis in microsporogenesis.

The degree of pollen sterility was lower than expected from the proportion of cells observed with bridges and fragments in anaphase I. However, this may have been due to differences in environment since pollen counts were made on field-grown plants and the cytological observations were on greenhouse grown plants. Swanson (19) in his study of inversions in *Tradescantia*, observed that the proportion of cells with bridges increased from 17.5 per cent to 47.8 per cent as the temperature during meiosis was increased from 12° C. to 27° C.

Inversions are widespread in nature, in both plants and animals. Various studies in *Drosophila* have revealed that collections of flies from different areas may differ only by the presence of an inversion in one of the chromosomes. In other words, inversions may be responsible for the appearance of races within a species.

The extensive breeding work in maize has brought to the attention of corn breeders and geneticists a large number of abnormal characters which had been carried in a hidden condition in the highly heterozygous open-pollinated varieties. Many of these characters are probably due to point mutations, but structural changes in the chromosomes may also be involved. On a few occasions, inbred lines have been found to be carrying interchanges which did not show up until crosses were made with a normal line. An inversion which causes no female sterility, as in the case of 6071, may not be detected. Actually, it may be of limited significance if the single cross of which it is one parent produces ample pollen when used as a male parent to produce double cross seed. However, crossing over in a long inversion may occur in 100 per cent of the cells resulting in close to 50 per cent of sterile pollen; or still more if other chromosomes contain inversions.

Possible uses of inversions in maize breeding problems have been discussed by Dobzhansky and Rhoades (9) and Sprague (16, 17). Dobzhansky and Rhoades have suggested using inversions to determine the yield genes carried on the various chromosomes of the different inbred lines. A homozygous inversion stock, with some suitable marker in the inverted segment, is crossed with an inbred line and the F_1 backcrossed to the inversion stock. The backcrossed progeny will consist of two types of plants, homozygous and heterozygous for the inversion. Presumably these two classes of plants differ only for the gene differences within the chromosome section marked by the inversion. As gametes resulting from crossing over within the inverted segment are mostly aborted, this provides a measure of the net effect of all dominant or partially dominant genes within this segment.

Sprague (16,17) has presented data on use of the inversion method indicating that it is feasible. A fifth chromosome inversion stock was used in which the inverted segment involved about 0.3 of the chromosome and was marked by the *pr* (red aleurone) colour factor. This was crossed with an inbred line *(Pr)* purple, backcrossed to the inversion stock *(pr)*, and the homozygous and heterozygous classes were compared for several characteristics. It was found that differences in yield per plot and weight per 500 kernels were significant. In each case the dominant factors were contributed by the dent corn inbred.

Sturtevant and Beadle (18) have discussed crossing over in included inversions in which a single crossover may give rise to a gamete which has a duplication for one or two genes but is otherwise normal. In plants as well as in *Drosophila* a gamete of this type may function in the production of a viable zygote. In plants, there is a possibility that such a duplication might be established in homozygous condition to increase the dosage of certain desirable genes, e.g. disease resistance or genes for sugary or other characters.

The use of structural changes such as translocations and inversions for breeding purposes is still in the experimental stages. Further investigations should give more detailed information on the possibilities of their uses. As more genes are located on the various chromosomes it will become easier to determine the breakage points of an inversion or other structural changes, and increase their usability by introducing marker genes.

SUMMARY

1. A cytogenetic study of a line of maize showing low pollen sterility but normally filled ears is described.

2. Cytological studies showed single bridges and fragments but no double bridges at anaphase I. There was a smaller number of cells with irregularities in division II. No bridges were observed at division II. Pachytene observations revealed the presence of loops, asynapsis, and reverse pairing in chromosome 2, similar to those expected from an inversion. One of the breaks was located at about 0.1 of the distance from the centromere to the end of the long arm, and the second break at about 0.6 of the long arm, near the pycnotic knob which is not included in the inversion. The total length of the inverted piece appears to have been about 50 per cent of the long arm.

3. The low pollen sterility due to the inversion was transmitted through both male and female. Progenies of crosses between normal and partially sterile plants varied rather widely in the proportions of low sterile plants,

the total for all the segregations being 49.3 per cent. The degree of pollen sterility varied from 12 to 22 per cent in different plants, but the average was 16.5 per cent.

4. A 5-point linkage test between genes in chromosome 2 and the low pollen sterility character associated with the heterozygous inversion indicated the order $lg - gl_2\ B - v_4 -$ sterility. Between v_4 and sterility the recombination value was 14.3 per cent. The recombination between sterility and colour booster *(B)* on the same chromosome but in the short arm was 34.5 per cent whereas between v_4 and *B* it was 29.0 per cent. A second 5-point test including *Ch* but not *B* gave 42.8 per cent recombination between sterility and *Ch* and 41.6 between v_4 and *Ch*. The gene, *Ch*, is probably located in the long arm of chromosome 2 distal to the inversion.

5. No reduction in genetic crossing over was observed in the inversion heterozygote, but the regions involved may have been too long.

6. It is suggested that eventually inversions may be of use in the production of stocks carrying a duplication for desirable genes.

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OBSERVATIONS ON SIX YEARS' SEED TREATMENT OF SOYBEANS IN ONTARIO[1]

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Until 1940 the average annual acreage of soybeans in Canada did not exceed 10,000 acres (2). By 1944, however, soybeans were grown in the Dominion to the extent of 45,000 acres (8) of which 44,700, yielding 845,000 bu. valued at $1,690,000, were grown in the Province of Ontario. Production in 1948 from a total of 94,000 acres (9) reached 1,683,000 bu. with a market value close to $4,000,000. Most of the soybean production in 1948, as in preceding years, was in southwestern Ontario.

During the period of rapidly-expanding acreage from 1942 until 1948, 14 parasitic diseases were reported by the present authors (5, 6) as occurring on soybeans in the high-production area of southwestern Ontario. Included among these were several that have been regarded by workers elsewhere as constituting a serious menace to the crop (7, 1). As a phase of the pathological investigations that have been carried out at Harrow in an endeavour to keep abreast of the potentially dangerous disease situation, a seed-treatment project, inaugurated in 1943, has been continued during the past six years. Originally, the experiment had been projected on a ten-year basis, but since the results indicate that little is to be gained by further repetition of the tests, the project has now been discontinued. A review of the investigations is presented in the present paper.

MATERIALS AND METHODS

Seed of A. K. Harrow, the variety best adapted to and, until recently, most extensively grown in the district, was used in all the tests. Each year an attempt was made to procure seed of as poor quality as possible, i.e., seed low in viability and high in disease potentiality. In some instances, such seed was readily available. For example, in 1942, due to extremely unfavourable weather conditions, soybean seed produced in southwestern Ontario was the poorest for many years. A sample selected for the 1943 spring trials showed a germinating capacity of only 23.4 per cent and a visibly diseased fraction of 40 per cent. In 1946, by examining seed lots from a number of different sources, it was possible to compound a sample containing 21 per cent definitely diseased seed, viz. 7 per cent mildew-encrusted (*Peronospora manshurica* (Naoumoff) Sydow), and 14 per cent "hilum canker", a condition characterized by a more or less distinct brownish-discoloured, canker-like injury immediately surrounding the hilum. The seed selected for the 1948 tests contained mildew-encrusted seed to the extent of 20.5 per cent. In 1944, 1945 and 1947, seed showing only a trace of disease had to be used.

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The protectants used, together with their composition and source, were as follows:

Spergon: tetrachloro para benzoquinone; Naugatuck Chemicals, Division of Dominion Rubber Company, Ltd., Elmira, Ontario.

Fermate: ferric dimethyldithiocarbamate, 70 per cent active material, wettable; Canadian Industries Limited, Chatham, Ontario.

Arasan: tetramethyl thiuramdisulphide, 50 per cent active material, non-wettable; Canadian Industries Limited, Chatham, Ontario.

Phygon: (later replaced by Phygon-XL): 2,3-dichloro-1,4 naphthoquinone; Naugatuck Chemicals, Division of Dominion Rubber Company, Ltd., Elmira, Ontario.

F-800: 2,4,5-trichloro phenyl chloroacetate; Dow Chemical Co., Midland, Michigan.

Seed lots after being treated early in February each year, at the rates indicated in Table 1, were packaged and stored in the laboratory until planting-time in May.

To promote natural infestation of the soil, the tests were carried out season after season in the same location. Each year, prior to planting, the soil, a Fox sandy loam, received an application of a 2-12-10 commercial fertilizer applied broadcast at the rate of 250 lb. per acre. All planting was done by hand in a randomized, 5-replicate design, the test unit within each plot comprising duplicate rows, 35 ft. long in 1943, 30 ft. from 1944 to 1948, inclusive. The rows, spaced 30 in. apart, were planted at the rate of 35 lb. per acre in 1943, and 30 lb. subsequently, the latter rate having been found by test to give maximum yield for the type of soil on which the experiment was being conducted. Planting dates were as follows: May 18 (1943); 15 (1944); 24 (1945); 13 (1946); 23 (1947), and 20 (1948).

Each year, counts made three weeks after planting furnished the data on emergence. Except for hand-weeding, cultural treatment of the plots conformed closely with accepted field practice for the crop. In harvesting, the plants were cut near the base of the stem with pruning shears, assembled in bundles, and threshed* the same day. All weighings were made immediately after threshing.

EXPERIMENTAL RESULTS

The results of the six years' experiments, in so far as emergence and yield are concerned, are summarized in Table 1. In regard to statistical treatment of data, the least significant difference between treatment means was determined for yield each year by the method of analysis of variance.

The results as shown in Table 1 are especially interesting for the first year's test in which 40 per cent diseased 1942 seed was used. In that particular test, treatment with Spergon resulted in an increase in yield that was highly significant over that resulting either from non-treated seed

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[*] 1943-1945, J. L. Jones, No. 4, pea and bean thresher; 1946-1948, portable, power-driven, rod-row thresher.

TABLE 1.—EMERGENCE AND YIELD IN RELATION TO DIFFERENCE IN QUALITY AND TREATMENT OF A. K. HARROW SOYBEAN SEED

Treatment		Year of test and quality of seed												5-Year average (1944-1948)	
Material	Rate of application	1943 40% diseased		1944 healthy		1945 healthy		1946 healthy		1947 21% diseased		1948 20.5% diseased			
		Em.* %	Bu. per ac.	Em. %	Bu. per ac.	Em. %	Bu. per ac.	Em. %	Bu. per ac.	Em. %	Bu. per ac.	Em. %	Bu. per ac.	Em. %	Bu. per ac.
Spergon	1943-44—2 oz. per bu. 1945-48—3 oz. per bu.	43.8	32.07†	94.3	26.76	78.9	26.47	89.7	26.28	93.2	24.48	91.1	37.36	89.4	28.36
Arasan	1943-44—1⅓ oz. per bu. 1945-48—2 oz. per bu.	17.0	23.82	84.2	26.85	79.6	25.07	90.8	25.89	93.7	23.99	91.2	39.25	89.9	28.21
Fermate	1943-48—2 oz. per bu.	15.0	24.81	84.7	27.34	80.0	27.20	91.3	27.68	94.2	23.79	90.4	38.72	88.1	28.94
Phygon	1946-47—4 oz. per bu. 1948 —3 oz. per bu.	—	—	—	—	—	—	84.8	24.73	92.1	23.06	87.3	35.42	88.0	27.73
Phygon-XL	1948 —3 oz. per bu.	—	—	—	—	—	—	—	—	—	—	89.5	37.46	—	—
F-800	1948 —2 oz. per bu.	—	—	—	—	—	—	—	—	—	—	80.3	37.21	—	—
CHECK		19.4	25.89	85.1	27.14	62.8	24.87	85.1	26.32	94.5	24.03	85.1	39.44	82.5	28.36

* Each year, the exact number of seeds planted was known; thus it was possible to express emergence on an absolute percentage basis.

† Highly significant over Arasan, Fermate and Check.

or from treatment with Arasan or with Fermate. In none of the other five tests, however, were comparable results obtained. Even in the case of the 1947 and 1948 seed, diseased to the extent of at least 20 per cent, yield differences between treatments were not significant and only in few instances did treatment give a possible significant increase in yield, e.g., Fermate over check in 1945. The average yields for treatments and check over the 5-year period 1944-1948 are surprisingly uniform.

More noteworthy than the yield differences between treatments or between treatments and check in any given year (except 1943) are those between different years. In the 1947 test, for example, yields were lower than for corresponding treatments in any other year. The reason for these lower yields is not difficult to explain. The mid- and later-summer season of 1947 was marked by a period of severe drought and high temperature, during which the experimental plots were extremely heavily infested by a species of red spider. The effects of the latter, superimposed upon those resulting from drought, undoubtedly were responsible for the reduced yields. Not so readily explainable, at first glance, however, are the tremendously increased yields in 1948, especially in view of the fact that, that year, fertilizer application was identical with and cultural treatments were similar to those of preceding seasons. Moreover, the increased yield cannot be explained on the basis of a difference in viability in favour of the seed used in 1948. Indeed, as Table 1 shows, emergence in 1948 was from 2 to 9 per cent lower than that in 1947. In a general way, it was known that the "weather" had been much more favourable for soybeans in 1948 than in certain, at least, of the preceding years. This circumstance emphasized the importance in such studies as these of careful consideration of "weather" in relation to other factors affecting emergence and yield. With this in mind, each year, for the period commencing May 10 and ending August 31, maximum and minimum daily temperatures, as well as rainfall, were plotted and expressed graphically as shown in Figure 1. Average seasonal yield for each treatment and check is also shown in the same figure.

Referring to Table 1, it will be noted that emergence in 1945 was generally lower than for any other year except 1943 when the extremely poor seed had been used. The planting date in 1945 was May 24. For an 11-day period just prior to that date, the minimum daily temperature ranged from 33° to 45° F., with an average of about 39° F. Then, following planting, for a 10-day period from May 29 until June 8, the minimum daily temperature again was low with an average of about 40° F. In no other year was the seed planted under such cold weather conditions. Since soil moisture was not lacking, the evidence strongly suggests that temperature was an important factor modifying emergence in 1945. Emergence for treated seed, it will be noted, averaged about 79 per cent, whereas that for untreated seed was 62.8 per cent. The increase of 16.2 per cent in favour of the treated seed would support the following statement made by Porter (10) in 1946: "Given unfavourable conditions for germination in the field, one could expect seed protectants to be of value even with seed of high vitality and if seed of low vitality were used the benefit should be greater." Unfortunately, increased emergence does not necessarily mean increased yields. This is clearly shown if emergence and yields for 1945 are compared

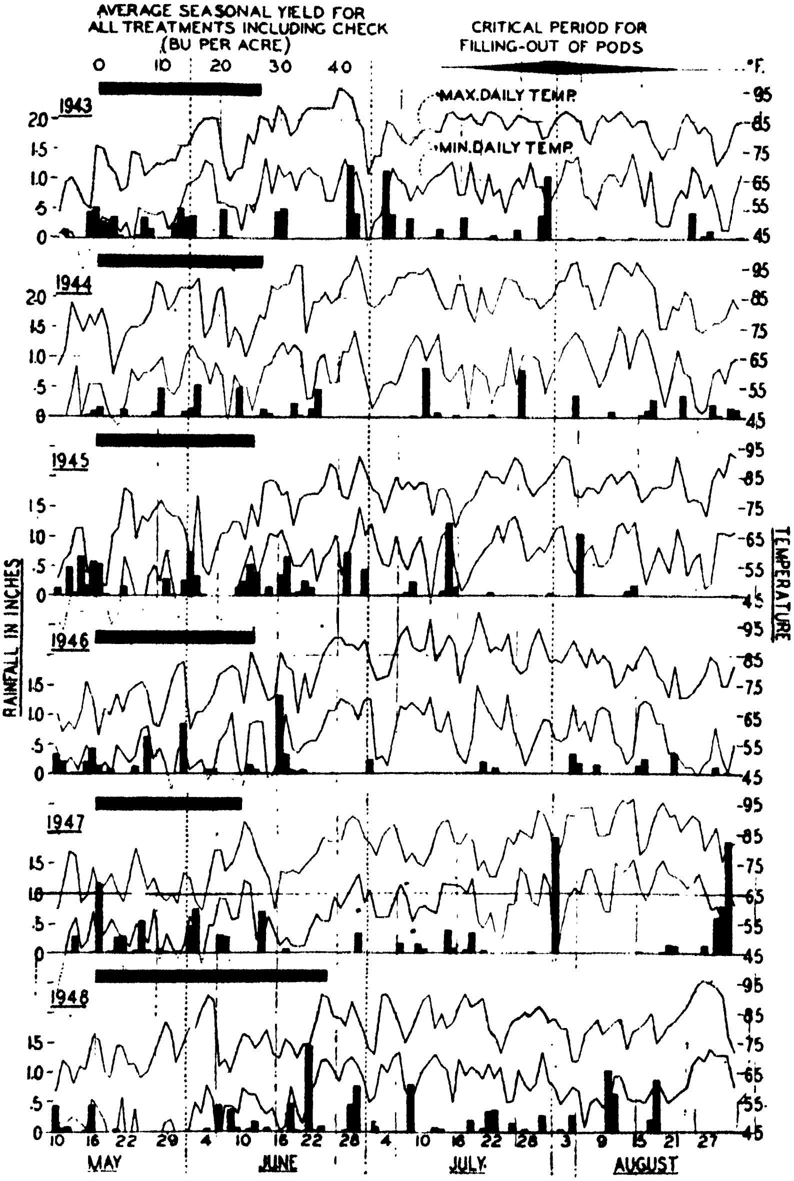

FIGURE 1. Seasonal rainfall and temperature in relation to yield of soybeans in experimental plots for the 6-year period 1943 to 1948, inclusive.

with those of 1944 and 1946. The question of the lack of correlation between emergence and yield has been discussed at length in a previous publication (5) and need not be dealt with further here. The point would seem to be established, however, that weather can be a factor in modifying yield even in the case of treated seed.

Later in the summer, when the pods are filling out, the weather is probably a more critical factor, as far as yield is concerned, than it is in the early growing season. In the Harrow area, the important period for pod and seed development extends from early in July until late in August. In examining the temperature conditions that prevailed during the 57-day period July 5 until August 31, for the different years of the test, it was found that the maximum temperature exceeded 85° F., on 21 days in 1943; 33 in 1944; 17 in 1945; 27 in 1946; 28 in 1947, and 16 in 1948. By this criterion it may be adjudged that, relatively, the mid-summer periods of 1944, 1946 and 1947 were hot; those of 1945 and 1948 appreciably cooler; while that of 1943 displayed a temperature intermediate between the extremes of the other years. If temperature were the chief limiting factor modifying yield, then it might be expected that yield difference could be correlated with temperature differences such as those just indicated. No such correlation exists, however, and temperature per se is ruled out as the important factor modifying yield. Of the remaining factors, soil moisture would present itself as the most important. During the period in question, i.e., July 5 until August 31, total precipitation for the different years was as follows: 1943, 3.33 in.; 1944, 3.68 in.; 1945, 3.24 in.; 1946, 1.84 in.; 1947, 6.97 in., and 1948, 4.52 in. If there were a direct correlation between amount of rainfall and yield, then yield should have been highest in 1947, intermediate in 1948, and lowest in 1946. No such gradation occurred, however, and it is obvious that, if a correlation between precipitation and yield does exist, it cannot be established by a consideration only of amount of rainfall. Distribution presents itself as a factor that must be taken into account. When Figure 1 is examined, it will be noted that, in 1948, for the 57-day period in question—and, in fact, for most of the growing season—rainfall was not only so adequate but was also so evenly distributed that a soil even as porous as the Fox sandy loam, on which the tests were being carried out, never approached the point of dryness at which growth of the soybeans might be adversely affected. Such was not the case, however, even in 1947 when, between July 5 and August 31, the rainfall of 6.97 in. exceeded by 2.45 in. that of 1948 (4.52 in.), for the same period. In 1947, as Figure 1 shows, most of the 6.97 in. came in almost cloud-burst proportions on August 1 and on August 30. Preceding August 1 and from that date until August 30, the weather was hot and dry, the soil dried out and for appreciably long periods the soybean plants showed severe wilting. In 1948, as has been pointed out above, the showers that produced 4.52 in. of rain, between July 5 and August 31, were ideally spaced and the plants never lacked adequate moisture. When additional comparisons are made between the rainfall of 1948 and that of 1944, 1945 and 1946, the importance of distribution of precipitation as a factor modifying yield becomes increasingly evident.

DISCUSSION

In 1947, in reporting the results of soybean seed treatment investigations up to that time, the present authors (5) stated that, "With poor quality, weather-damaged seed . . . . and with the cracked-coat fraction of an otherwise high-quality seed . . . . treatment with Spergon increased emergence and yield. These were the only instances, however, in which increases in early stands of plants as the result of seed treatment were correlated with statistically significant increases in yield." It was further pointed out that these results were substantially in accord with those reported by several contemporary workers in the United States, all of whom had found that, although seed treatment (not necessarily with Spergon in all cases) might give increased stands over non-treated checks, yields in general remained unaffected. As the result of more recent work as reported not only here by the present authors but also by investigators in the United States (3, 4, 11), there would seem to be no reason for modifying the earlier view that if high-germination, good quality soybean seed is planted, seed treatment is of doubtful value.

SUMMARY

From 1943 to 1948, inclusive, soybean seed of the variety A. K. Harrow, varying from year to year both as to germinability and disease potentiality, was treated with Spergon, Arasan, Fermate, Phygon, Phygon-XL and F-800 and planted in outdoor plots in a randomized, 5-replicate design. In the case of the extremely poor-quality 1943 seed (germinating capacity 23.4 per cent and visibly diseased to the extent of 40 per cent), treatment with Spergon increased emergence and yield. This was the only instance, however, in which an increase in early stand of plants, as the result of seed treatment, was correlated with a statistically significant increase in yield.

More noteworthy than the effect of seed treatment in modifying yield was that of favourable weather, a remarkable increase of some 10 bu. per acre in 1948 over all other years, apparently being correlated more closely with an adequate, well-distributed rainfall than with any other factor.

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ROLLER CRUSHER FOR DRYING HAY

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Means of suitably curing or drying forage crops is a major problem in farm operation. Numerous methods and various types of equipment have been used in an attempt to protect the hay crop from the weather and to decrease the length of time that this crop normally remains in the field during curing and harvesting operations. The crushing of green hay by a machine consisting primarily of two steel rollers is one device which has been used in an endeavour to increase the rate of drying or curing hay. To determine the value of this machine in haying operations, experiments have been conducted at the Central Experimental Farm, Ottawa, in which the rate of drying of crushed hay was compared with uncrushed hay.

The crusher used in the tests consists of two steel rollers, six inches in diameter and five feet long, a pick-up device for lifting the hay into the rollers, and an auxiliary engine for driving the rollers. Two springs are used to maintain a pressure between the rolls and the speed of the rolls is approximately 250 revolutions per minute.

In principle, the machine is designed to sufficiently compress a swath of hay as it passes between the rolls to crack the stems of plants and to bruise the leaves. It is not designed to squeeze or press moisture out of

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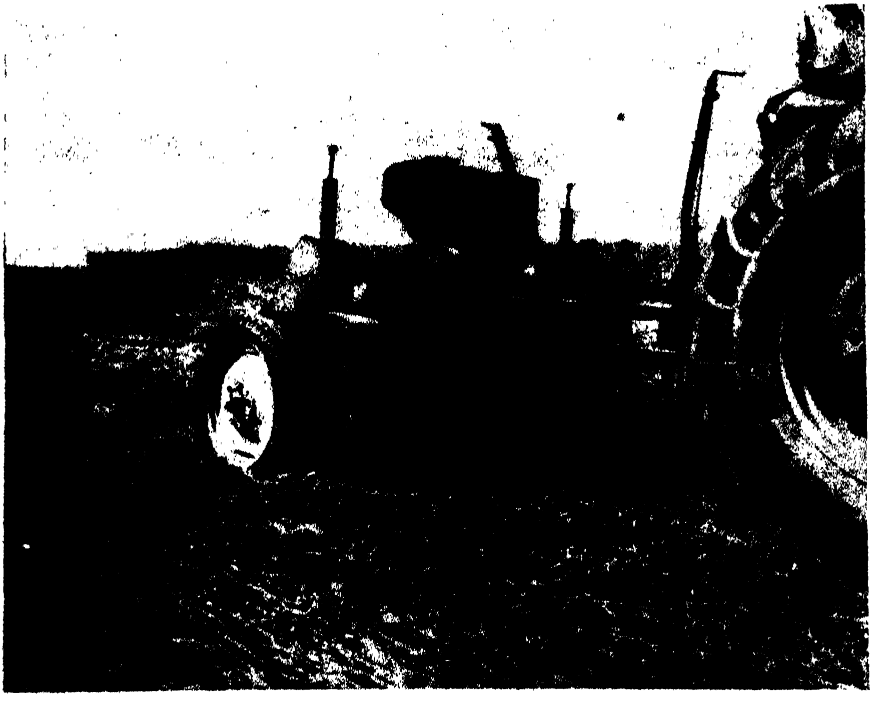


FIGURE 1. Front view of crusher

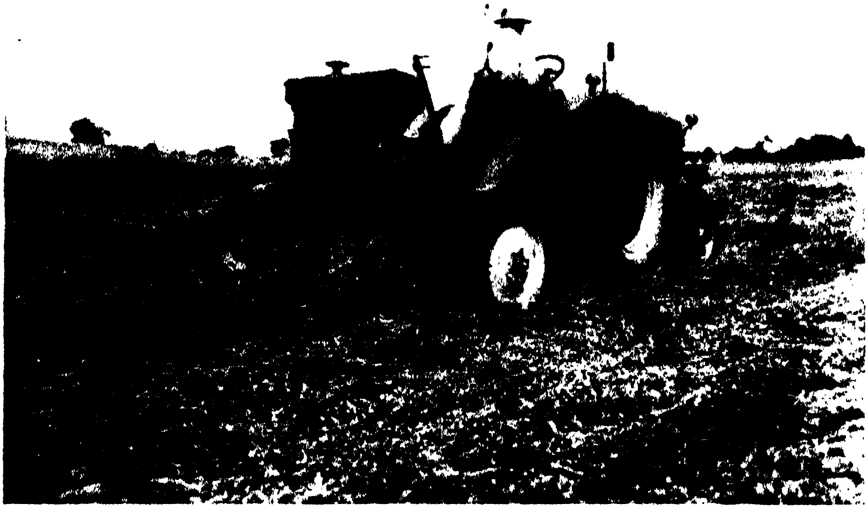


FIGURE 2. Rear view of crusher

the plants as might be expected; but there is some free moisture present on the compressed hay due to bruising of both the stem and leaf. The action of the machine is intended to increase the rate of drying of the stems of the plants in order that the stem might dry at approximately the same rate as the leaf.

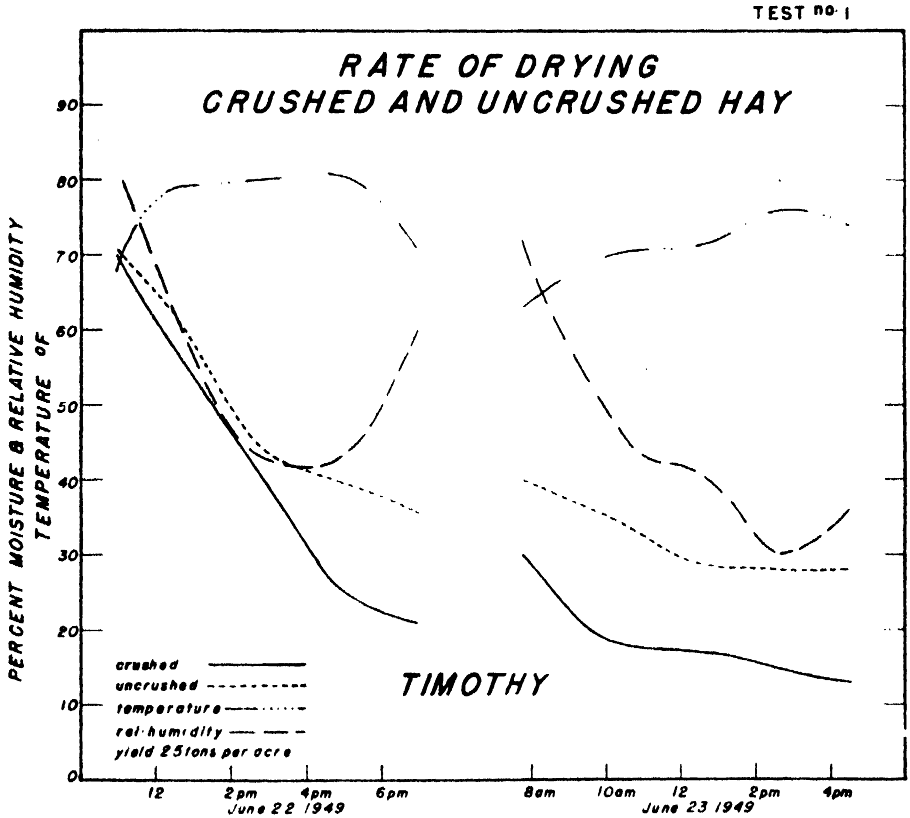
Field units for the crushing of hay did not originate recently; some units were built prior to 1937, and the idea may have been conceived many years earlier. However, the mechanism of the first machines was not well developed and they were not produced or available in any quantity for farm use.

EXPERIMENTS ON CRUSHED AND UNCRUSHED HAY

Field tests were conducted on hay crops consisting of first cut timothy, alfalfa and clover (3 to 2), alfalfa and timothy (3 to 1) and second-cut clover. Every other swath in the field was crushed while the intermediate swaths served as a check. Replicate samples of hay were taken to determine the rate of drying of the crushed and uncrushed material. Other data, including temperature and relative humidity, were noted and plotted on the attached charts. All tests were performed when the weather was favourable for curing hay.

Test No. 1

This test was conducted on a crop of timothy that was cut approximately two days before full bloom and which yielded 2.5 tons per acre. The crop was cut at 8.30 a.m. and the moisture content of the crop was



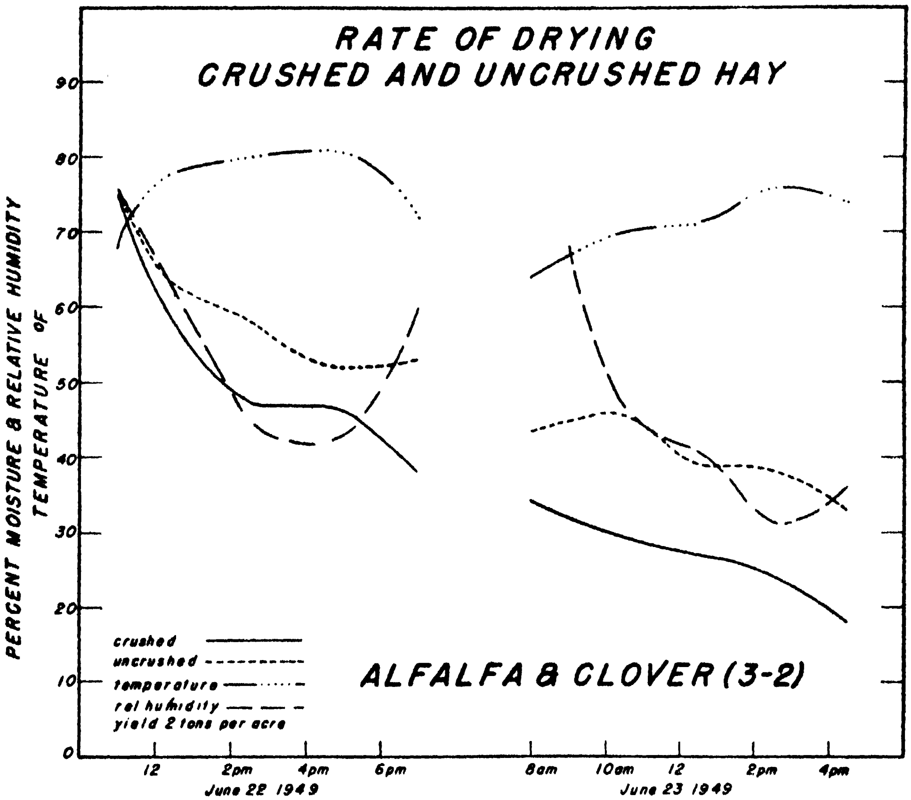
70 per cent when it was crushed at 11 a.m. From the first graph it may be noted that the crushed material reached 25 per cent moisture at 5 p.m. in the afternoon and could have been hauled to storage at this time while the uncrushed material reached a moisture content of only 45 per cent. Both the crushed and uncrushed material gathered moisture during the night.

At 9 a.m. of the second day the crushed material was at 25 per cent or 11 per cent lower than the uncrushed material, and thus again suitable for storage. The hay in the field was raked at 3 p.m. and the uncrushed crop was suitable for storage at approximately 11 a.m. of the third day.

Test No. 2

In this test the crop used was a mixture of alfalfa and clover (3 to 2) that yielded two tons per acre and which was cut at full bloom. On the day the crop was cut the initial moisture was 75 per cent. By 7 p.m. oven-dried samples indicated a moisture content of 54 per cent for uncrushed material and 36.5 per cent for the crushed hay. At 2 p.m. the following day the crushed alfalfa and clover was below 25 per cent and suitable for storage while the uncrushed hay had reached a moisture of 33 per cent. An upward trend in the moisture content curve for uncrushed hay between 5 p.m. and 7 p.m. on June 22 has been attributed to difficulties in obtaining representative samples of the hay as it cured in the swath.

TEST no 2



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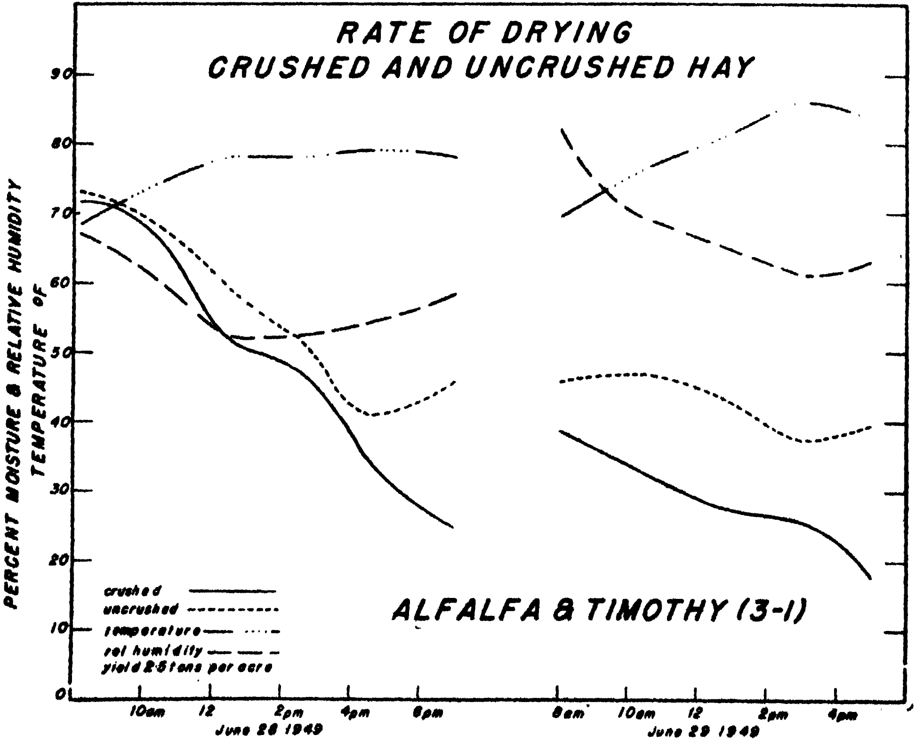

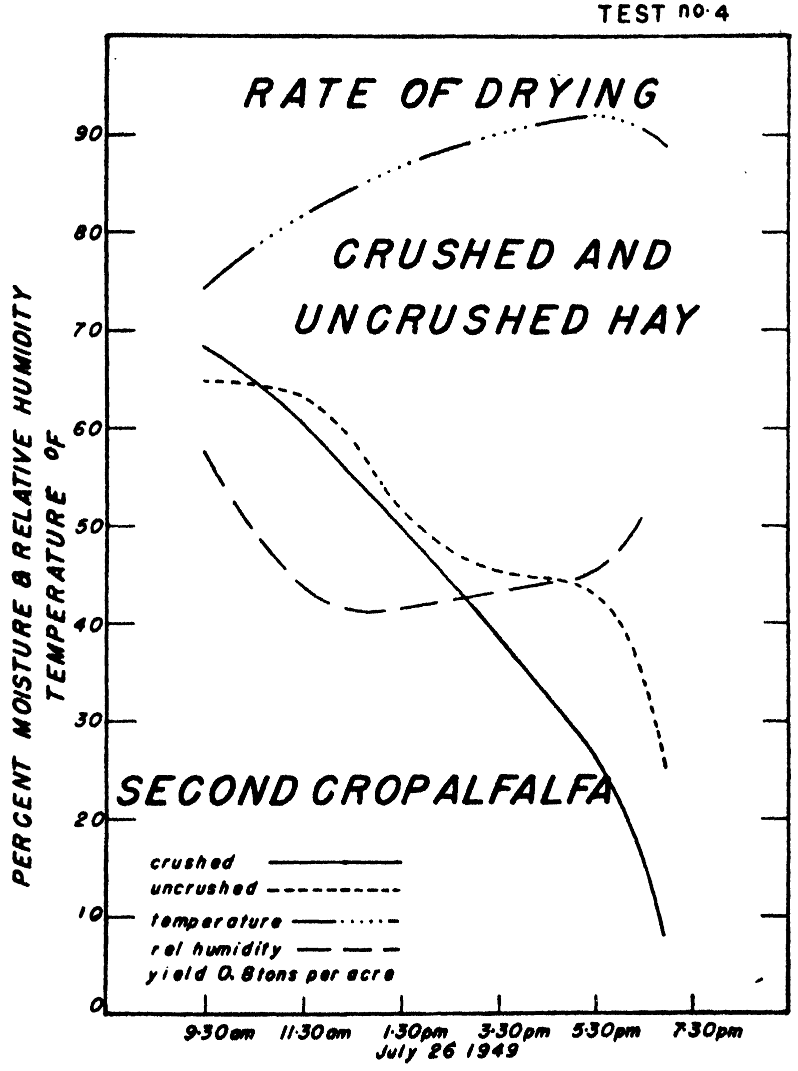

Test No. 3

The crop used for field test No. 3 was an alfalfa timothy mixture (3 to 1) with the alfalfa at three-quarter bloom and the timothy at full bloom. This crop yielded 2 5 tons per acre. The graph for alfalfa and timothy shows that the crushed hay was sufficiently dry for storage at 7 p.m. on the day it was cut, while the other material contained 45 per cent moisture. As the crushed hay could not be hauled from the field that evening, it gathered moisture during the night and was not stored until after noon on the following day, while the uncrushed hay could not be placed in the barn until one day later. In this test the uncrushed material did not show any increase in moisture content during the night. As both crushed and uncrushed hay normally gather moisture overnight this apparent discrepancy could only be attributed to a sampling error. Even by taking a considerable number of samples of hay as it cures in the field, and by attempting to secure representative samples, discrepancies of this nature were not entirely eliminated.

Test No. 4

Second-cut alfalfa yielding 0.8 tons per acre was used in the final test. Due to the dry season this crop was poor with the plant height ranging from 4 to 14 inches. The initial moisture content was below the other crops tested. The crushed alfalfa dropped to a moisture content of 25 per cent by 5.30 p.m., and the uncrushed reached this moisture content at 7 o'clock of the same evening. Due to this light yield, high temperatures, low relative humidity and good air circulation there was very little difference between the crushed and uncrushed samples. On the second day both samples were below 20 per cent moisture content; therefore, testing was discontinued.

SUMMARY

In the three tests on first-cut crops of hay the crusher operated in a fully satisfactory manner. On the second-cut crop, which was short and particularly low in yield, a considerable amount of difficulty was experienced with the machine. Even though the speed of travel was varied from three to five miles per hour, the pressure on the rolls was varied, and other adjustments made, it was not possible to obtain an even flow of the material through the crusher rolls.

The tests indicate that there is a closer relationship between the rate of drying and air humidity for uncrushed than for crushed hay. It may be noted from the graphs that uncrushed or ordinary hay started to absorb moisture sooner than the crushed hay when the humidity began to increase during the late afternoon and evening.

In general, it appears that crushing of the hay is beneficial for early cut hay, which has a reasonably high yield and which contains a considerable amount of moisture at the time of cutting. Three tests show that during favourable weather crushed hay cut in the morning was suitable for storage by the evening of the same day, and that this hay could be conveniently stored at noon on the following day. Uncrushed hay required two days to cure under the same conditions and could only be hauled to storage at noon on the third day. This represents a saving of time of one day in three days under favourable weather conditions.

According to experiments conducted by Jones (1), the crushed method proved more beneficial on crops or plants having thick heavy succulent stems and leaves than on other crops. In the above tests there was no significant difference between timothy and alfalfa clover mixtures. Feeding tests were not conducted on the crushed and uncrushed hay at Ottawa, but Pennsylvania State College (2) reports no significant difference in the feeding value of the hay which has been crushed and hay that is cured in the regular manner.

In studying the data presented for this experiment it should be noted that results are based only on tests in 1949 when the weather was favourable for curing hay.

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PRELIMINARY STUDIES ON A PYTHIUM ROOTROT OF SPANISH ONION SEEDLINGS[1]

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INTRODUCTION

In recent years a serious rootrot of Spanish onion seedlings has been observed in numerous greenhouses in southwestern Ontario. Because of the yellowish discoloration of the leaves of affected seedlings and of the occurrence of the latter in scattered groups in the flats, in which they are commonly grown, the trouble has come to be known locally as "Yellow Patch".

The disease has been severe whether the seed has been planted in steamed or in non-steamed soil. Although the number of seedlings affected varies from flat to flat in any given planting, the trouble develops in a high percentage of flats in every greenhouse previous to the transplanting of the onions to the field. By mid-April, at the usual time for transplanting, seedlings are often so stunted and unthrifty, as a result of extensive root injury, that frequently less than 50 per cent are large enough to be set in the field. Because of the consistent occurrence of this trouble it ranks in economic importance as the most serious seedling disease of Spanish onions in southern Ontario. The purpose of this preliminary paper is to report the cause of the disease.

LITERATURE REVIEW

No extensive investigations of rootrot of Spanish onion seedlings appear to have been conducted hitherto. In 1948, however, Semeniuk (3) briefly mentioned a *Pythium* seedling rot and root necrosis of *Allium cepa*. By plating diseased roots on agar, the investigator consistently obtained a high percentage of *Pythium* species, including *P. irregulare*, *P. ultimum*, *P. spinosum*, *P. mamillatum*, *P. debaryanum* and *P. graminicolum*. He also found Fusarium developing from nearly every root and less frequently *Phoma terrestris*, a chlamydosporous *Phoma* sp., and other fungi. According to this author, symptoms of *Pythium* parasitism are seed rotting, pre-emergence seedling rot, stunted emerged seedlings, which often die, seedling damping-off, and lead-coloured or faintly brown, yellowish or pink roots lacking cortex in advanced stages of disease.

DESCRIPTION OF THE DISEASE

The presence of the disease is first noticed about 4 to 7 weeks after seeding and becomes increasingly apparent after the seedlings have been "topped" two or three times. While all the plants in a flat are occasionally attacked, more often, in the incipient stages at least, as the name "Yellow Patch" implies, only part of a flat is affected.

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The conspicuous symptoms are wilting accompanied by yellowing of the tips of the younger leaves and complete blanching and ultimately death of the older leaves. Affected seedlings are severely stunted and exhibit characteristic symptoms of starvation and drought.

Observations in commercial greenhouses indicate that seedlings attacked after they are four or more weeks old are seldom killed outright. Ordinarily, when conditions favour good growth, late-infected seedlings have been observed to recover if left in the original flats for two to three weeks after the usual transplanting time. However, since late plantings result in greatly reduced yields, such recovered plants are of little value.

When diseased plants are carefully pulled from the soil and washed in water, many of the oldest roots are observed to be severely decayed and often only a few fragments of root tissue remain attached to the base of the bulb (Figure 1). Characteristically, the root system manifests an advanced stage of necrosis before above-ground symptoms are very conspicuous (Figures 8 and 9). Concurrent with the rotting of the oldest roots is a tendency for the plant to "bulb" (Figure 1) and to produce new adventitious roots. Plants six or more weeks old have often furnished evidence of having lost roots continuously and yet have continued to produce new roots. The epidermal tissue of the bulbous part of affected onions is frequently brown and necrotic.

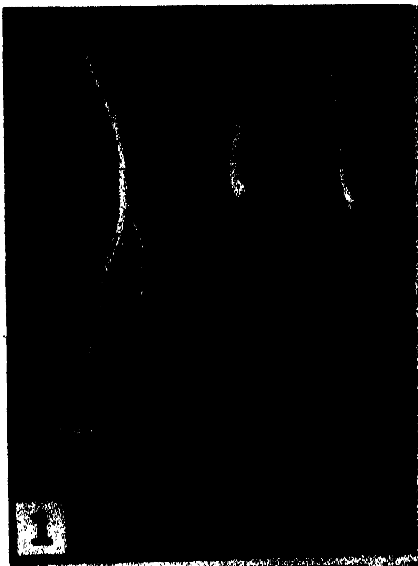
When affected roots are immersed in water they are found to be glassy and transparent in appearance. In incipient lesions, however, it has been observed that the vascular cylinder remains as a white strand for some time after the cortical cells have become completely transparent. Furthermore, this sleeve of necrotic, colourless, cortical tissue can be readily pulled from the root with dissecting needles. Older roots, when attacked, become yellow or faintly brown in colour before they collapse. In general, root necrosis develops rapidly.

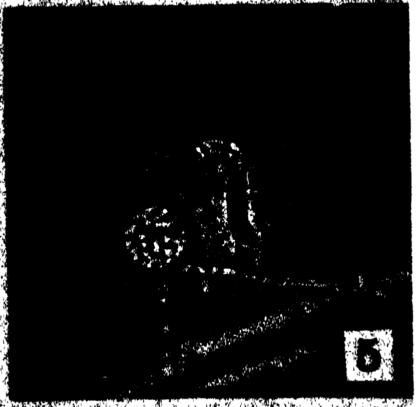
Microscopic examination of necrotic roots consistently revealed the presence of oospores throughout the root (Figure 2), indicating the invasion of all tissues. The epidermal tissue of the bulb is also invaded by the fungus. Oospores may occur singly or may be aggregated in a cell. In the advancing margin of the lesion both fungus mycelium and sporangia are readily observed. In addition, nematodes are found to be frequent invaders of necrotic roots.

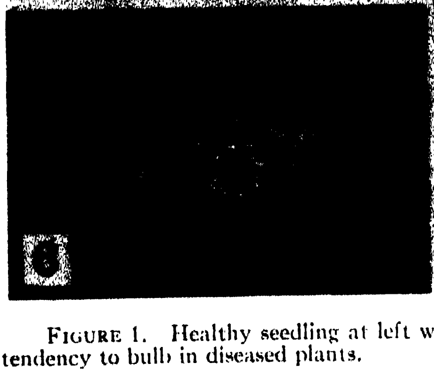
ISOLATIONS FROM DISEASED ROOTS

During the course of the investigation more than 300 root fragments of diseased onion seedlings obtained from several greenhouses were plated on potato dextrose agar. In a high percentage of the earlier isolations, in which diseased tissue, after being surface-sterilized in 1/1000 mercuric chloride solution, was plated on acidified agar, the following organisms were obtained: *Fusarium* sp., *Mucor* sp., *Penicillium* sp., and *Rhizoctonia Solani.* In 90 per cent of the later isolations in which surface sterilization was not used and in which the root fragments, after having been thoroughly rinsed in several transfers of sterile water, were buried in non-acidified agar, a *Pythium* sp. was obtained. Because of the consistency of occurrence of the last-mentioned fungus it was tested for pathogenicity and cultured for identification.

PLATE I







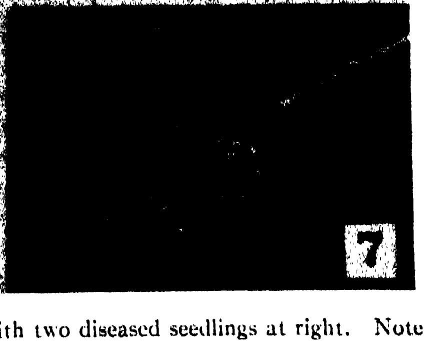


FIGURE 1. Healthy seedling at left with two diseased seedlings at right. Note tendency to bulb in diseased plants.

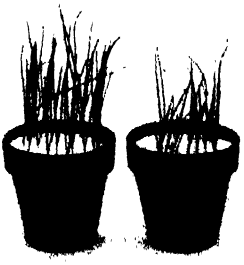
FIGURE 2. Oospores of *Pythium irregulare* in cortical cells of onion root (X350).

FIGURE 3. Oogonium in necrotic root showing protuberance on oogonial wall (X760).

FIGURE 4. Sexual organs just prior to fertilization. Note the long oogonial projection and the long falcate antheridial branch (X760).

FIGURE 5. Sexual organs just after fertilization (X760).

FIGURES 6 and 7. Stages of sexual reproduction. Note the short oogonial projections in Figure 6 and the longer septate one in Figure 7 (X760).



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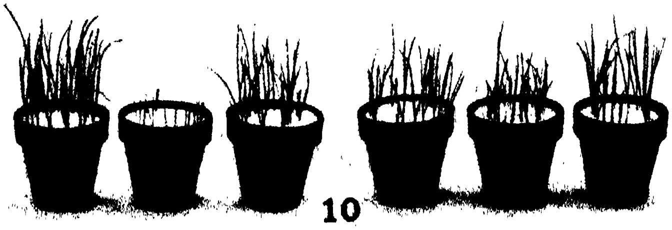

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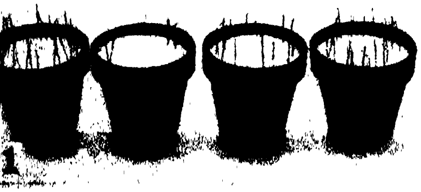

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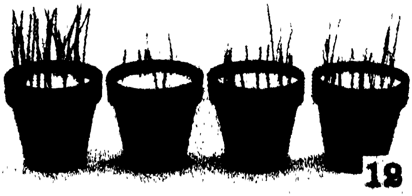

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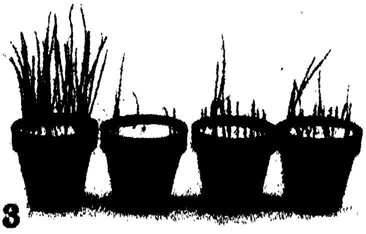

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FIGURE 8. Six-week-old Spanish onion seedlings; non-inoculated control at left, seedlings in pot at right were inoculated with *P. irregulare* 17 days previously.

FIGURE 9. The inverted contents of two pots shown in Figure 8. Note the white mass of roots in check at left and scarcely visible, necrotic roots of inoculated seedlings at right.

FIGURE 10. Six-week-old onion seedlings inoculated at different ages with *P. irregulare*. Left to right are check, inoculated at planting, at emergence, at 7, 14, and 21 days after emergence. Toothpicks represent killed seedlings.

FIGURE 11. Sixteen-day-old onion seedlings grown in steam sterilized soil where *P. irregulare* was placed at various levels below the seed. Left to right, check, inoculum placed ½, 1½, and 3 inches below seed. Toothpicks represent killed seedlings.

FIGURES 12 and 13. Same as Figure 11 showing seedlings at 4 and 5½ weeks old, respectively.

PATHOGENICITY

A preliminary test was conducted in the greenhouse in mid-July by infecting steamed soil in which the onion seedlings were growing with agar cultures of the *Pythium* isolate. The fungus was observed to invade the roots of the young seedlings and to cause some necrosis. Attacked plants, however, continued to grow rapidly and manifested no conspicuous above-ground symptoms. In further tests in which the fungus was introduced into vials of nutrient solution where 17-day old seedlings were being grown, the roots were observed to be invaded immediately. All root tissues were then extensively parasitized and the seedlings were killed in a few days.

IDENTIFICATION AND DESCRIPTION OF THE PATHOGEN

When the fungus was grown on potato dextrose agar, sporangia were observed to develop in four to five days and sexual organs (Figures 4, 5, 6, and 7) in five to seven days. In gross morphology and in measurements of size of sporangia, oogonia, and oospores the fungus was indistinguishable from *Pythium irregulare* Buisman as described by Middleton (2).

The sporangia show great diversity in shape, manifesting spherical, elliptical and pyriform types. Spherical to lemon-shaped oogonia are usually intercalary and often possess protuberances that are readily found when the fungus is grown on potato dextrose agar but are only rarely seen in necrotic root tissue (Figure 3). When a small piece of agar upon which the fungus was growing actively was transferred to sterile water in a Petri plate, the nature of the oogonial protuberances could be very clearly seen as further growth was made. The projections are few to numerous, of irregular length and are also irregularly located on the oogonial wall. Long projections are occasionally found to be septate (Figure 7). Antheridia are usually monoclinous, occasionally diclinous, rarely more than three per oogonium and more often one or two. Considerable variation was found both in the length and shape of the antheridial branch. The antheridial cell is typically short, slightly swollen, crook-necked and borne on a slightly curved stalk (Figures 5, 6, and 7). Occasionally, however, the antheridial cell is long, allantoid-clavate and is borne on a relatively long, sometimes 100 μ, falcate stalk (Figure 4). The oospores are aplerotic. Germination was not observed.

INFECTION EXPERIMENTS

. In order to study the pathogenicity of the fungus under conditions of growth similar to those existing in commercial plantings two experiments were conducted in the greenhouse in early spring.

Effect of Pythium Irregulare Upon Spanish Onion Seedlings When Placed at Three Different Depths Below the Seed

Entire Petri-dish cultures of *Pythium irregulare,* grown on potato dextrose agar, were placed in steam-sterilized soil in 4-inch pots at the following depths: ½ inch, 1½ inches and 3 inches. To ensure actively-growing mycelium, 2-day old cultures of the fungus were employed in this experiment. Twenty-five Spanish onion seeds were sown in a circle towards the circumference of each pot and were covered with three-eighths inches of soil.

TABLE 1.—SEEDLING EMERGENCE AND POST-EMERGENCE MORTALITY OF SPANISH ONIONS GROWN IN STEAMED SOIL WITH *Pythium irregulare* PLACED AT THREE DEPTHS BELOW THE SEED

Depth of infestation (inches)	Percentage				
	Emergence	Post-emergence mortality* at end of:			Stand 6 weeks after seeding
		2 weeks	3 weeks	5 weeks	
¼	36.0	7.4	84.0	88.8	4.0
1½	76.0	17.0	50.0	70.1	27.4
3	89.3	0.0	12.4	30.6	62.0
Check	86.0	0.0	0.0	0.0	86.0

* Post-emergence mortality is shown as a percentage of the emergence.

TABLE 2.—WEIGHTS OF TOPS AND ROOTS OF SPANISH ONION SEEDLINGS GROWN IN STEAMED SOIL IN WHICH *Pythium irregulare* WAS PLACED AT THREE DEPTHS BELOW THE SEED

Depth of infestation (inches)	Total number of plants	Average weight in grams*		
		Entire plant	Tops	Roots
¼	6	0.34	0.21	0.13
1½	29	0.63	0.46	0.17
3	91	0.42	0.30	0.12
Check	124	0.89	0.54	0.35

* Average of six pots six weeks after seeding.

The experiment provided six replications for each depth of infestation with a similar number of pots containing sterile agar to serve as controls. The soil in the pots was kept moist by sprinkling with water daily. The test was carried out in the greenhouse in late February, March and early April under environmental conditions similar to those obtaining in commercial greenhouses. The seedlings were "topped" three times during the course of the experiment. The seedling emergence and post-emergence mortality was recorded periodically and results are presented in Table 1. Six weeks after seeding all living plants were removed from the pots, washed free from soil, the tops and roots were air-dried and then weighed separately. These weights are shown in Table 2.

If reference is made to Table 1, it will be noted that, when the inoculum was placed ¼ inch and 1½ inches below the seed, the emergence was 40 per cent and 10 per cent lower, respectively, than in the check. There was, however, no decrease in emergence when the fungus was placed at the 3-inch level. For the most part, decrease in emergence in the inoculated soils has been considered to be due to pre-emergence damping-off. *Pythium irregulare* caused post-emergence killing at all three depths of infestation (Figures 11, 12, and 13). This manifestation of the disease was not only more severe at the ¼ and 1½-inch levels than at the 3-inch level, but appeared earlier and typical damping-off, as expressed by a collapse of tissue at the soil line, developed. At the 3-inch level of inoculation, however, post-emergence killing was characterized by a progressive stunting, wilting and yellowing of above-ground parts until finally, death ensued. On such plants most of the roots were found to be completely rotted soon after the first appearance of symptoms on the leaves. In many of these plants, even though extensive root necrosis developed, new roots were produced beyond the diseased area at the periphery of the bulb. Moreover, partial recovery of many affected seedlings was observed at the end of the experiment. As revealed by Table 1, in the aggregate, the closer the inoculum was placed to the seed the lesser was the stand obtained.

The weight of roots and tops was in agreement with the appearance of the plants. As Table 2 shows, the average weights of the roots and tops in the check were appreciably greater than in any of the inoculated pots. If, during the course of the experiment, "topping" had not been practised, the weight of the non-inoculated controls would have undoubtedly been still greater because more leaf growth was removed from these plants than from the inoculated plants.

Effect of Pythium Irregulare Upon Spanish Onion Seedlings of Different Ages

Since the disease appears characteristically in commercial greenhouses, where the seed is sown in steamed soil, not at the time of emergence but after the crop has made considerable growth, it would seem that the fungus enters the soil some time after the seedlings emerge and subsequently builds up rapidly. Furthermore, the results of the previous experiment, where steamed soil was infected at different depths, suggested that seedlings were susceptible for a considerable time. To ascertain the probable time of infection, plants of different ages were exposed to *Pythium irregulare* in the following manner: A 1-inch core of inoculum was inserted in a hole three inches deep in the centre of 4-inch pots of steamed soil at the time of seeding, at emergence, at 7, 14, and 21 days after emergence. The inoculum consisted of a 4 per cent mixture, by weight, of cornmeal in sand through which the fungus had completely grown. Sterile cornmeal-sand medium was added to the check pots. Twenty-five seeds were planted in a circle toward the outside of each pot and were covered with three-eighths inches of soil. In this experiment six pots comprised the test unit for each of the five inoculations and the uninoculated check. The tops of the plants were "cut back" three times during the course of the experiment. As a final measure of the effects of the fungus, all plants were removed from the pots at the end of six weeks, washed free from soil, air-dried, and the roots and tops weighed separately. Results are listed below in Table 3.

As Table 3 shows, post-emergence mortality was found to be extensive only when the fungus was introduced into the soil at the time of seeding. Seedlings that were killed showed typical symptoms of damping-off, i.e. a collapse of tissues at the ground level. All seedlings that were exposed to inoculum at the time of emergence and at later stages manifested wilting and yellowing of leaves 14 to 27 days after inoculation. The later appearance of symptoms in plants inoculated at emergence and at seven days' post-emergence than in those inoculated subsequently may have resulted from the use of inoculum which was 21 and 28 days old, respectively, in the former, whereas for all other inoculations 12- and 14-day old inoculum was used.

TABLE 3.—THE RELATION OF AGE OF SPANISH ONION SEEDLINGS GROWN IN STEAMED SOIL INOCULATED WITH *Pythium irregulare* TO THE TIME OF SYMPTOM DEVELOPMENT, POST-EMERGENCE MORTALITY, AND WEIGHT OF ROOTS AND TOPS

Time of inoculation	Percentage post-emergence mortality*	Period for symptom appearance in days**	Total number of plants	Average weight in grams		
				Entire plant	Tops	Roots
At seeding	65.0	—	23	0.46	0.31	0.15
At emergence	7.3	24	97	0.55	0.35	0.20
7 days post-emergence	1.3	27	108	0.52	0.34	0.18
14 days post-emergence	0.6	14	104	0.37	0.27	0.10
21 days post-emergence	0.0	15	111	0.41	0.30	0.11
Check	0.0	—	110	0.75	0.43	0.32

* Post-emergence mortality is shown as a percentage of the emergence.

** Period for symptom appearance is the time in days between inoculation and the first evidence of disease on above-ground parts.

Concomitant with the appearance of above-ground symptoms was a typical root necrosis. As mentioned previously, in many diseased seedlings, at least 50 per cent of the roots were rotted before any conspicuous above-ground evidence of disease was noted. The fungus was readily isolated from these necrotic roots.

As expressed in Table 3, the average weight of roots and tops was found to be considerably greater in the checks than in any of the inoculated units. This finding was in accord with the general appearance of the plants (Figure 10).

CONTROL OF THE DISEASE

In a preliminary experiment in which Arasan (50 per cent tetramethyl thiuram disulphide) was added to steam-sterilized soil, at the rate of 3.1 gm. per cu. foot, previous to seeding, evidence of the effectiveness of the treatment in the control of rootrot was obtained. This rate of application not only delayed the appearance of the disease but appreciably reduced the incidence of rootrot. Further research is being done on this phase of the disease and will be reported in a later publication.

SUMMARY AND CONCLUSIONS

In recent years a rootrot of Spanish onion seedlings, grown either in steamed or in non-steamed soil in greenhouse flats, has become of economic importance in southern Ontario. The disease appears characteristically from four to seven weeks after planting, and causes stunting, wilting, and yellowing of leaves associated with extensive root necrosis.

Isolations from diseased seedlings revealed the consistent presence of a fungus identified as *Pythium irregulare* Buis.

In infection experiments, *Pythium irregulare* was shown to be highly pathogenic to Spanish onion seedlings, causing both pre-emergence and post-emergence damping-off. The attack of older seedlings was characterized by virtually complete necrosis of existing roots coincident with wilting, yellowing, stunting, and occasionally death of all above-ground parts. In these experiments, *P. irregulare* clearly duplicated the symptoms observed in large commercial plantings and may be considered the chief causal agent. Hence, it would appear that the pathogen invades the steamed soil in the onion flats a few weeks after seeding and that the disease develops rather quickly under normal conditions of growth.

Results of a preliminary experiment showed that Arasan (50 per cent tetramethyl thiuram disulphide), when added to steamed soil at the rate of 3.1 gm. per cu. foot, effected considerable control of the disease.

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FIELD OBSERVATIONS ON THE BLACK ARMY CUTWORM, *ACTEBIA FENNICA* (TAUSCH.), AND ITS CONTROL ON BLUEBERRIES IN NEW BRUNSWICK

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During the seasons of 1944 and 1945 the black army cutworm, *Actebia fennica* (Tausch.), destroyed many hundreds of acres of commercially grown blueberries and seriously damaged many more in Charlotte County, New Brunswick, the losses amounting to thousands of dollars. In the same years, blueberries in the State of Maine suffered very seriously from its depredations. Growers in Charlotte County had not previously experienced damage by this insect although it was reported as a pest of blueberries in Maine in 1925 by Phipps (1).

LIFE-HISTORY

As the buds of the blueberry begin to swell in early spring, the overwintering larvae emerge from the soil, crawl up the blueberry stems, and begin boring into the buds. At this time the larvae are from one-quarter to one-half of an inch in length. Rarely a very small larva has been found within a bud, suggesting that it had overwintered in the egg stage. The egg, however, has not been seen. Full growth is reached early in June, when the insect pupates in the soil to emerge as an adult in late July or early August.

NATURE OF INJURY

Very severe but inconspicuous damage may be caused to the swelling buds by the young larva, which bores through the sides of the bud scales to eat out the interior. The small, round hole in the side of the bud is not readily seen, and estimation of the prevalence of the insect in a large barren is difficult in the early stages of plant growth. Occasionally a bud scale drops from an injured bud, producing a one-sided appearance which is more easily noted. Squeezing a bud gently between the fingers will readily indicate whether it is infested. As the buds burst, injured areas of the barren become at once apparent by contrast with the greener, uninjured areas.

The larva remains hidden in the black ash and duff on the surface of the soil of the barrens during the daytime, and emerges to crawl up the stems and feed shortly after sunset. Blossom buds and open blossoms are preferred to the foliage. Usually the terminal buds are attacked first, the larva feeding on the remaining buds as it descends. Practically all damage caused during the two years of the outbreak was on areas in their first year of fruit production after the "burn". Areas in production two or more years following burning suffered very little from this insect. Indications are that the moths invariably lay their eggs on areas in "sprouts", i.e., on areas burned over in the early spring of that year.

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CHARACTERISTICS OF THE OUTBREAK

The first known infestation of the 1944 outbreak was reported on May 24 on a barren in heavy full bloom. All of the blossoms on this and many other barrens were devoured two to three days later, and the green foliage was also consumed before the caterpillars pupated on May 28 and 29. On many barrens the caterpillars began marching when most of the foliage had been stripped but the marching suddenly ceased at pupation. Complete stripping occurred on many barrens in the two main producing areas of the county, one being a narrow coastal strip some 20 miles in length near Pennfield, and the other an inland area between Whittier Ridge and Tower Hill, roughly 12 miles long and five miles wide.

In 1945 the outbreak occurred in the same areas, again on barrens which were in the first year of fruit production, and which had been burned over in the spring of 1944. On smooth barrens, i.e., areas which at one time were cultivated, infestations were more or less evenly distributed throughout the area. On rough barrens moderate infestations were confined to the hummocks and knolls, the low and wet places being left uninjured. However, stripping occurred in heavy infestations on rough barrens.

During the major part of the larval period in both years of the outbreak, weather conditions were abnormal. Records for May in the two years are compared below with the average for the previous 31 years:

	Precipitation	Mean temperature	Total sunshine
	inches	° F.	hours
1944	2.08	55.0	290
1945	8.61	47.9	165
Average (31 years)	2.86	47.9	205

Although conditions for plant and insect development in 1944 must have been ideal, it is probable that the rate of development of the blueberry was more rapid than that of the cutworm, so that the plants produced heavy bloom before defoliation occurred. Under the cold and wet conditions of 1945 both blueberry and cutworm growth rates were retarded, resulting in the destruction of blossom buds with little or no production of bloom in some areas. Cutworms collected in the field early in 1945 and reared in the insectary increased in size much more rapidly than those in the field.

ESTIMATION OF PREVALENCE OF THE CUTWORM

After the experience of the 1944 outbreak it was necessary as soon as possible in the spring of 1945 to discover the distribution and prevalence of the insect in many barrens over a wide area so that measures for control could be begun before serious damage occurred.

The careful examination of individual buds before they burst is time-consuming and does not accurately indicate the location and degree of infestations. Searching the duff and debris on the surface of the soil with the forefinger or a small whisk-broom will usually result in the discovery of a few cutworms in heavy infestations; but again the distribution and population of the insect cannot be determined. Phipps (loc. cit.) remarks that the grower "should visit his field with a flashlight the next warm night in order to see the worms actually at work", and remarks further that they are conspicuous. The writer found that the cutworms fed readily at temperatures as low as 38° F. during either wet or fine weather. He also noted that, though examination of the barrens with a flashlight showed many larvae feeding, many were missed because the dark colours of the worm blended with the black ash of the barren.

It was found that sweeping the areas at night with a heavy sweeping bag quickly resulted in accurate data on the location and degree of infestation. The bag was made of heavy cotton, 30 inches deep and 18 inches in diameter, and mounted on a stiff wire hoop with a handle five feet long. Sweepings made on many barrens gave data indicating that economic losses should not occur in infestations totalling fewer than 15 worms per 50 sweeps. When the total exceeded 25 worms per 50 sweeps the immediate adoption of control measures was indicated.

CONTROL EXPERIMENTS

In 1945, dusting experiments were carried out in various areas having heavy infestations as indicated by sweeping tests. Results were recorded by noting the number of cutworms collected per 50 sweeps in the various plots. The actual counting of cutworms on all plots was shortly discontinued, however, as being time-consuming and of no significant value; for all plots, with the exception of those treated with DDT, yielded collections of hundreds of cutworms per 50 sweeps, indicating no apparent control.

Four series of 0.2-acre plots were treated at different dates, the amounts per acre being indicated in brackets: 3 per cent DDT (70 lb.); 2 per cent Lethane (70 lb.); copper sulphate-calcium arsenate-hydrated lime, 20-20-60 (60 lb.); gypsum-synthetic cryolite, 70-30 (60 lb.). The first treatment (Series 1) was applied on May 9. A very heavy and untimely snow-storm two days later covered the plots to a depth of 10 inches. On May 14, as soon as the snow had melted, another series of plots (Series 2) were treated and test sweepings were made on Series 1. One sweeping of 50 strokes down the centre of each plot yielded hundreds of cutworms from all except the DDT plot; none was collected in this plot although it was swept many times. The second and two more series (Series 3 and 4), dusted on May 16 and 20, gave identical results, namely, almost 100 per cent mortality in the DDT plot and no apparent control from any other treatment. Twenty-four hours after the treatment of Series 3, great numbers of cutworms were found violently wriggling in the black ash and duff on the DDT plot. The affected worms which were collected died within 24 hours. On May 21, 3 per cent DDT dust at 40 lb. per acre and also undiluted cryolite at 30 lb. were applied on replicated plots from 1 to 3 acres in extent. The DDT again produced almost 100 per cent mortality, whereas cryolite did not give any apparent reduction.

Tests were carried out with poisoned bran bait made according to the following formula:

Wheat bran	20 pounds
Paris green	1 pound
Molasses	2 quarts

The bait was applied at 40 lb. per acre without producing any obvious effect on the cutworm population. Substitution of oil (Imperial White Oil No. 5, Visc. Saybolt 81) for the molasses did not increase the effectiveness of the bait. Many growers used the bran bait at amounts ranging from 20 to 150 lb. per acre. Sweepings on some of the treated areas, including one huge barren where the bait had been applied three times, gave large collections of cutworms, indicating little or no control.

CONCLUSIONS

1. Sweeping of the blueberry barren at night before damage becomes apparent is the most satisfactory method of determining the possible need for control of the black army cutworm.

2. Three per cent DDT dust, used at the rate of 40 lb. per acre, gave almost 100 per cent mortality of the insect. Amounts as low as 25 lb. per acre may give economic control.

3. Two per cent Lethane, 20 per cent calcium arsenate, and synthetic cryolite dusts were ineffective.

4. The application of poisoned bran bait in amounts up to 150 lb. per acre provided no appreciable control. The substitution of molasses by a light oil did not increase the effectiveness of the bait.

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BOOK REVIEW

VERNALIZATION AND PHOTOPERIODISM, by A. E. Murneek and R. O. Whyte with H. A. Allard, H. A. Borthwick, Erwin Bünning, G. L. Funke, Karl C. Hamner, S. B. Hendricks, A. Lang, M. Y. Nuttonson, M. W. Parker, R. H. Roberts, S. M. Sircar, B. Esther Struckmeyer, and F. W. Went. Foreword by Kenneth V. Thimann. Waltham, Mass.: Chronica Botanica Co.; Ottawa: Thorburn and Abbott, Ltd. XV + 196 pages, illus. $4.50.

This symposium is of great value to all interested in the study of plant growth. The authors are well known to agronomists and plant physiologists. At the end of each section there is a comprehensive list of references and literature is cited freely throughout the text. A number of subjects are dealt with but they are allied and interdependent.

In the first section, theories dealing with low temperature treatment of seeds and the resulting responses have been clarified. The principle is emphasized that, if germinating seeds respond to low temperatures, a high or an alternating temperature and treatment in the absence of 0_2 may reverse or arrest the process of vernalization. The second section deals with photoperiodism as modified by temperature and other environmental factors. The following six sections are chiefly concerned with hormones, wave lengths, nutrition and metabolism, as a means of explaining vernalization and photoperiodism in relation to flower formation. In many plants definite anatomical and histological changes occur as the plants approach the cycle of sexual maturity. Length of day in climates is regarded as a major factor in the distribution and changing of flora throughout the history of this planet. The three remaining sections, aside from the final special supplement, discuss vernalization and photoperiodism in the tropics and make preliminary observations on phenological data as a tool in the study of photoperiodic and thermal requirements of plants and the thermoperiodicity of light and temperature optima at various stages of plant growth. The special supplement in German contains studies of photoperiodicity in the tropics, the significance of daily rhythm of endogenous plants, and genetic studies of photoperiodism in crosses between Mammoth (short day) and Java (day-neutral) tobaccos. A study of this book will assist the agronomist and physiologist to assess and interpret responses of plant growth to the environment. It is recommended as a valuable guide and reference book.

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USE OF THE SURVEY METHOD IN HORTICULTURAL RESEARCH[1]

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PURPOSE OF A HORTICULTURAL SURVEY

The survey method has been widely used in horticultural research. Little appears to have been published, however, on the specific advantages and disadvantages of this method of horticultural research, as compared with more exact methods. This paper constitutes a discussion of the method and its usefulness, as revealed in several investigations conducted by the author and his co-workers.

The purpose of a survey can be twofold. First, it can be used to determine the numerical distribution of classes of individuals. For example, a survey might be conducted to determine the number of fruit trees of each kind and variety that are present in certain areas at any one time. It could be amplified to include such factors as size of tree, condition of tree, yield, etc. The major purpose, however, would be a mere recording of facts. It would be necessary either to record every individual tree in the areas concerned, or to sample the orchards or the trees in a representative manner, so that the data obtained could be considered a reliable index of the total.

In the second place, the survey method can be used for the purpose of determining relationships between factors and functions, between causes and effects. For example, it may be desired to determine whether any relationship exists between soil texture (a factor) and yield of apple trees (a function) under field conditions, or between the crop on a tree (factor) and the length of the terminal shoots the same year (function). The major purpose would be to discover more-or-less fundamental laws or relationships that could be applied at any time under the same or similar circumstances. It would not be necessary to sample the available population in such a manner as to represent the whole population proportionately; in some cases at least, it would probably be more important to make certain that the samples be taken so as to represent a wide range of each of the factors under consideration. For example, in studying the relationships between soil texture and tree yield, it would seem advisable to take texture and yield records under a wide range of soil textural classes. This type of survey may not be considered a survey in the true sense of the word. There does not appear to be any better word, however, to describe it.

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These two purposes of a survey can be accomplished separately or together. In the investigations that form the basis of this discussion, the second purpose was the primary one; and any information obtained that would satisfy the first purpose can be considered only incidental.

Whatever the purpose of a field survey, it is usually necessary to sample a comparatively large number of individuals. For example, it would not be sufficient to record the yield of one apple tree growing on each of five soil textures, and to come to conclusions on the basis of this sample. Perhaps a total of 100 or more trees would be required. As will be made clear in the discussion to follow, the reasons for this are field variability and the large number of factors concerned.

SOURCES OF MATERIAL

The sources of material that are used as a basis for this discussion are as follows:—

1. Studies of the relationships between, and the value of, different methods of expressing tree vigour. In a series of investigations, the relationships among trunk circumference, annual increase in trunk circumference, terminal shoot length and diameter, percentages of bloom and set of fruit, and yield, were studied over a period of years on apple trees. The initial results, on 58 McIntosh trees, were reported in 1937 (2, 3, 4).

2. Studies of the relationships between field measurements and laboratory measurements of soil moisture, using 100 soil samples collected at the field capacity for moisture in several soil series. Data were obtained, on each sample, of moisture holding capacity, wilting coefficient, available moisture content, percentages of sand, silt, clay and colloid, and settling volume. The results were reported in 1941 (6, 7). A second investigation of similar nature, using 93 soil samples, was reported in 1949 (14).

3. Studies of some factors affecting apple yields. Mature McIntosh trees were selected in different soil series in different districts in the Okanagan Valley, to a total of 400 trees in 85 groups of five or less. This was later reduced to 290 trees in 73 groups or plots. A deliberate attempt was made to include wide variations in soil texture, soil depth, fertilizer treatment and cultural methods. Soil and twig samples were analysed for N, P, K, Ca and Mg; determinations were made of the pH, salt content, lime content, organic matter content, and sand, silt, clay and colloid contents of the soil; root counts were made; and records were taken over a six-year period on tree size, tree spacing, increase in trunk circumference, terminal length, yield and grade of fruit. The relationships among most of these factors have now been studied, and have been reported in a series of papers (5, 8, 9, 10, 11, 12, 13). The findings of this investigation have provided the major source of material for the present discussion.

In all of these investigations, the relationships among the various sets of records have been determined primarily by means of the coefficient of correlation. Scatter diagrams have been freely used to assist in explaining the correlations. In many cases, the effects of one or more factors on a correlation have been eliminated by calculation of partial coefficients of correlation. Where it has appeared advisable to eliminate the effects of more than two factors (as in the third investigation), coefficients of regression have been determined, the yield or other function has been corrected tree by tree for variations in each factor in turn, and the adjusted yields have then been correlated with the next factor under consideration. In some cases, the analysis of covariance procedure has been used (1). These procedures will be referred to further in the discussion below.

SOME PROBLEMS ENCOUNTERED

Many problems were encountered in the investigations noted above. Some of these problems were solved, others were not. Among the more difficult problems were those associated with the selection of materials, the sampling, measuring and recording, and the interpretation of results.

Selection of Materials

It was recognized at the start of each investigation that each measurement would be affected by a large number of factors, and that it would be advisable to select the materials in such a manner as to eliminate as many as possible of those factors not pertinent to the investigation. This was especially true in the apple investigation. Here all nutritional factors were considered to be of especial interest, while other factors were not; hence it was decided to record all nutritional factors as far as time and expense would permit, and where feasible to ignore the other factors.

It was not found feasible, in the apple investigation, to record all of the nutritional factors. This was due partly to lack of time and partly to the fact that there were too many nutritional factors to be studied all at once. Some were ignored, (such as minor elements), on the assumption that they were unimportant; but whether or not they were actually unimportant was not determined. One serious problem was encountered in connection with the soil moisture status. An attempt was made to eliminate any major effects of soil moisture by selecting only orchards where the moisture content was maintained at "adequate" levels, i.e. between field capacity and the wilting point. Although a careful watch was maintained on irrigation procedures, it appeared quite possible that adequate soil moisture contents were actually not maintained in every case.

Neither was it found feasible, in the apple investigation, to ignore all of the non-nutritional factors. It was foreseen that some of them would have major effects on the growth and yield of the trees. In certain cases, it was found possible to measure some of these factors, as in the case of size of tree and distance of planting. In other instances, an attempt was made to minimize the effects of the factors in the selection of material. For example, only one variety of apple was used, the McIntosh; only trees that appeared to be in good health were selected, thus minimizing the effects of diseases, insect pests, and winter injury; and only those orchards were selected in which the trees appeared to receive good care as far as thinning, pruning, spraying, etc., were concerned. These precautions assisted greatly in reducing the effects of non-nutritional factors. However, during the course of the investigation it became apparent that the yield or quality of fruit in many cases was being reduced by crown rot, oyster-shell scale, leaf hoppers, poor pruning, poor thinning or other factors. Where this was recognized, such trees in most cases were eliminated from the experiment, so that the original 400 trees were reduced to 290. It is quite possible that in many cases the yield or quality was reduced by factors not measured and not even recognized.

In the slection of materials, it was considered advisable to include a wide range of each of the major factors under consideration. For example, in the apple investigation, groups of trees were located in a wide range of soil textures, soil depths, and fertilizer treatments. This did not constitute a serious problem. In the other investigations, also, no serious difficulty was encountered in obtaining a wide range of the factors being studied.

Recording and Sampling

Owing to the field variability encountered, it was not considered necessary to be absolutely accurate in the recording of the field data. In the apple investigation, for example, yields were recorded in bushel boxes, to the nearest half-box; shoot growth was added on a steel tape, and the total per tree recorded to the nearest centimetre; and tree spacing was measured to the nearest foot. The results obtained indicated that additional accuracy would have been superfluous.

Because of the many factors affecting fruit tree performance, it was considered advisable to select a large number of trees on which to take records. This increased greatly the work of recording and sampling. It was not found possible always to be at the right place at the right time. The chief difficulty encountered in this respect was in recording the yield per tree, as the operator could not be present in all 73 plots throughout the length of the Okanagan Valley at the same time; hence it was necessary to obtain the assistance of the fruit-growers in the recording. For the most part they did a good job, but in some cases they did not. Errors in recording of the yield were revealed by individual tree estimates made beforehand; nevertheless, this did constitute a source of error. It was found necessary to discard some of the yield records.

One of the greatest sources of error in field work is inadequate sampling, especially of the soil and of plant tissues. In the apple investigation, soil samples were taken around an individual tree in each plot, each composite being obtained from ten locations. This was adequate for these individual trees, but not always for the whole plots. Soil variations were sometimes great within individual plots.

Interpretation of Results

The interpretation of results is one of the most difficult steps in an investigation of this nature. The difficulty arises chiefly from the large number of factors entering into the results. This holds true even when some factors have been largely eliminated by care in the selection of material. In these investigations, some of the factors affecting the results have been measured, some have not. When they have not been measured, there has been no way of adjusting for their variability. And even when they have been measured, their effects on one another and on the function (e.g. yield) have been so interrelated and complex that satisfactory segregation of individual relationships has sometimes been almost impossible.

The correlation procedure, supported by scatter diagrams, has formed the statistical basis for the studies discussed herein. In some cases, simple correlations gave the desired information. For example, in the first soil moisture studies the correlation between percentage colloid in the soil and the moisture holding capacity was + 0·886**[1], a very high figure for this type of material. There could be no doubting the fact that a direct relationship existed. In the apple investigation, the correlation between size of tree and biennial bearing index was only + 0·049, which provided no evidence of any relationship. In such cases, a decision as to significance was comparatively easy. In other cases, however, it was not so easy.

It was obvious from field observation and from the correlations, that there were many cases of two or more interrelated factors affecting the same function. In such cases the partial correlation method was used to eliminate the effects of one or two factors at a time. For example, in the terminal shoot studies a correlation of + 0·853** was obtained between terminal diameter and terminal length, and + 0·558** between terminal diameter and annual trunk increase. The correlation between trunk increase and terminal length was + 0·380**. By the partial correlation procedure, the first two correlation figures became + 0·836** and + 0·487** respectively. This did not change the picture greatly. In other cases, however, the picture was quite definitely changed. For example, the correlation between soil pH and apple yield was + 0·267*, indicating a definite relationship between the two. Both of these were also related to the moisture holding capacity (correlations + 0·586** and + 0·410** respectively). When the effects of moisture-holding capacity were eliminated, the correlation between soil pH and yield was reduced to + 0·036. This indicated the possibility that the apparent effects of pH on yield may have been incidental to the more general effects of soil texture on yield. It illustrated one of the pitfalls encountered in interpreting correlations.

In the apple investigation, too many factors were measured to allow elimination by the partial correlation procedure. At the same time, it was considered necessary to eliminate some of them statistically in order to study the others. Three factors were selected that showed close relationships with yield, these being size of tree, terminal length, and biennial bearing index. The regression of yield on size of tree was calculated, and all yields were individually adjusted to a standard size of tree. The regression of this adjusted yield on terminal length was then calculated and the procedure repeated. The same was then done with the biennial bearing index. The other factors were then correlated with these triply-adjusted yields, and in some cases also with the unadjusted, the singly-adjusted and the doubly-adjusted yields. This procedure revealed several relationships that could not otherwise have readily been discovered. With so many adjustments, however, the interaction of the factors sometimes made interpretation of the results difficult. It became obvious as the investigation progressed that only those correlations with high significance could be accepted as being valid. It also became obvious that in an investigation of this kind the more factors that could be eliminated in the initial selection of material, the more reliable would be those results obtained from the factors recorded.

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[1] Two asterisks following a coefficient of correlation indicate odds greater than 99:1 (highly significant). One asterisk represents odds between 99:1 and 19:1 (significant). And no asterisk represents odds below 19:1 (non-significant).

The question arises as to whether factors other than those recorded and used for adjustment purposes might not reduce the tree yields to such an extent as to mask the effects of the factors recorded. This no doubt had some effect on the factors of minor importance; that is, those which had a lesser effect on the function, as, for example, the P or K content of the shoots in the apple investigation. With factors of major importance it reduced the coefficients of correlation considerably but did not entirely mask the relationship. In the case of the effects of soil texture on tree yield (see Figure 1) it is obvious that the lower yields were reduced by factors other than soil texture. The upper yields across the top of the diagram were probably reduced little, if at all, and appear to represent reasonably well the trend in these selected plots. The trend for the whole distribution, however, is not so greatly different from that of the higher yielding plots, and the coefficient of correlation ($+ 0 \cdot 410^{**}$) is still considered to be "highly significant". The same holds true with the correlation between the biennial bearing index and tree yield (see Figure 2).

Several of the correlations were deceptive. Results that appeared at first to be clear-cut were found, on further investigation, to be misleading. For example, in the tree vigour studies a correlation of $+ 0 \cdot 437^{**}$ was ob-obtained between increase in trunk circumference and terminal length in 1934; and one of $+ 0 \cdot 526^{**}$ was obtained in 1935. It appeared from this that not only did the more vigorous trees grow more in both these ways, over a period of years, than did the less vigorous trees, but they must also have been affected in similar manner by biennial bearing. This latter, however, did not prove to be the case, as was shown by further studies. In order to determine the effects of biennial bearing on annual measurements, it was found necessary to eliminate the effects of general differences in tree vigour. This was accomplished by determining the 1934/1935 ratios for both increase in trunk circumference and terminal length. When these ratios were correlated with one another the coefficient was $- 0 \cdot 417^{**}$. This opposite effect of biennial bearing on these two factors has been found to hold true in all subsequent investigations.

Another example of a deceptive relationship was that obtained between the K content of the soil and yield. A coefficient of $+ 0 \cdot 374^{**}$ was obtained, indicating that higher yields were, on the average, obtained where the soils contained higher percentages of available K. As with the case of soil pH noted above, however, both the K content and the yield were closely correlated with the soil texture as represented by the moisture-holding capacity. When the effects of moisture-holding capacity were eliminated, the partial correlation obtained was only $+ 0 \cdot 032$. It might be concluded from this that there actually was no relationship between K content of soil and yield. Even this conclusion, however, might not be justified, as there remains the possibility that the beneficial effects of heavier soils on yield might have been due in part to their higher contents of available K. This illustrates well the confusion that may arise in interpreting results.

A correlation is, of course, merely a mathematical expression of a relationship between two sets of data. Either one of these may be a factor and the other a function; or both may be functions affected in similar or in opposite manner by a common set of factors. For example, a correlation of $+0{\cdot}410^{**}$ between moisture-holding capacity of the soil and tree yield merely indicates that from the statistical viewpoint, there is a highly significant positive relationship between the two sets of data. It remains for the operator to suggest which affects which, if either, or whether the relationship is merely coincidental. In this case the moisture-holding capacity of the soil was considered to be the factor, and yield the function. It could not be concluded, however, that the moisture-holding capacity had a direct positive effect on yield. All that could be concluded was that the two were closely related, and that possibly soil moisture, colloid content, nutrient content, and indeed all those factors associated with soil texture, were instrumental in affecting the yield. In the same manner, caution had to be exercised in interpreting many of the other correlations. Another example was the very high correlation ($-0{\cdot}914^{**}$) between the sand content of the soil and its wilting coefficient. It could be concluded that the sand in the soil had an inverse or negative effect on the wilting coefficient; but it would appear to be more correct to say that the silt + clay content, which supplements the sand content, has a direct or positive effect on the wilting coefficient.

In this discussion of some of the problems encountered, not only a number of the difficulties, but also some of the methods used in overcoming these difficulties have been reviewed. All of the major difficulties cannot be overcome under field conditions; but in spite of this it may be possible to glean sufficient information from a survey to make the investigation well worth-while.

FINDINGS OF VALUE

The above discussion of the many difficulties encountered in these investigations could well leave the impression that the investigations were not worth-while. In order to balance the picture somewhat, therefore, the major findings will be briefly reviewed.

Tree Vigour Studies

High positive correlations were obtained between terminal length, terminal diameter and increase in trunk circumference. The diameter near the tip of the terminal was found to be better than that at the base. As the trees increased in size, their vigour tended to decrease, although this was not considered to be a fundamental relationship, as it depended largely on the amount of nitrogen applied. With trees bearing biennially, the year of heavy crop was the year of smaller increase in trunk circumference but longer terminals. These findings were very valuable in developing simple methods of measuring the vigour of mature bearing trees.

Soil Moisture Studies

In the first investigation, correlations were obtained between the silt, clay, and colloid contents of the soil and the settling volume on the one hand, and field capacity, wilting coefficient and available moisture content of the soil on the other hand. Straight regression lines were calculated for use in determining the three moisture contents from mechanical analysis or settling volume measurements. The errors involved in making such determinations were also calculated. The same types of correlation were obtained in a second investigation, in which the moisture equivalents were also determined. Curved regression lines were calculated this time, and the errors involved in using them were likewise determined. The most reliable laboratory methods for determining the three pertinent moisture contents of the soil were presented. These results have been of great value in subsequent field studies involving soil moisture, and in the classification of soils on the basis of their moisture-holding capacity.

Apple Yield Studies

The findings noted above on tree vigour were confirmed. Close positive correlations were obtained over a period of years between increase in trunk circumference, terminal length, and terminal diameter. With biennially bearing trees the trunks grew more and the terminals were shorter but thicker in the "off" year than in the "on" year. High positive correlations were obtained over a period of years between tree size and terminal length on the one hand, and total yield and yield of high quality fruit ("profitable yield") on the other hand. A still higher negative correlation was obtained between the degree of biennial bearing and yield. An increase in the degree of biennial bearing was accompanied by a greater reduction in yield than was found by variations in any other single factor studied in this investigation. The optimum degree of biennial bearing was found to be zero, and the optimum annual terminal growth 25 centimetres.

The heavier soils were found to have higher pH values than the lighter soils, also higher contents of organic matter, lime, and available calcium, potassium and magnesium. There was no clear relationship between soil texture and P content. The heavier the soil, the more vigorous were the trees and the higher was their yield; just what were the causes of these relationships was not determined. Higher yields were also obtained with higher pH values and higher contents of organic matter, K, Ca and Mg in the soil, though it was questionable whether any of these factors had any actual measurable effect on tree yield. A negative relationship was found between soil pH and P content, and positive relationships between soil pH and contents of K, Ca and Mg. No clear-cut relationship was found between root distribution and tree growth or yield.

By analysis of the terminal shoots and leaves, it was found that in the "on" year the N content was lower and the P and K contents were higher than in the "off" year. This finding was important in affecting the method of selecting samples for tissue analysis. Positive correlations were obtained between the available P and K contents of the soil and the P and K contents of the shoots; between the N and P contents of the shoots; between N content of shoots and terminal length, and between N content of shoots and yield. Correlations between P or K content and yield were not significant.

One of the main purposes of this investigation was to find out if any major nutrients were deficient in any part of the Okanagan Valley. Nitrogen was the only element studied that was found to be definitely deficient in any of the plots. If deficiencies of P, K, Ca or Mg occurred, it was not possible to establish these deficiencies definitely by this procedure. Possibly some deficiencies were present, but not in sufficient degree to affect tree growth and yield adversely; that is, adversely enough to produce statistically significant correlations between the nutrient contents of the soil or terminal

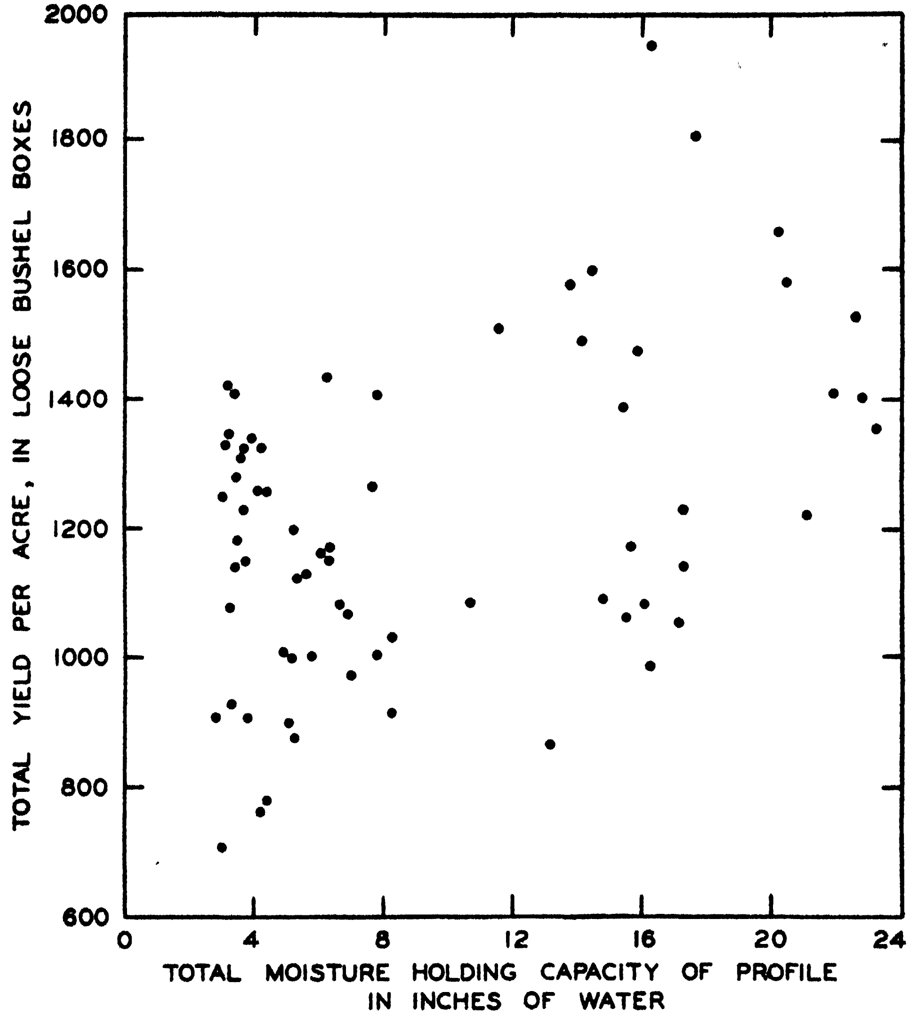

FIGURE 1.—Scatter diagram of total moisture holding capacity of profile charted against total yield per acre. r = + 0·410

shoot and tree growth or yield. It is also possible that definite deficiencies did occur, but that their presence was masked by factors which had a much stronger influence on growth and yield.

These investigations have served to indicate the value of measurement and statistical analysis as compared with observation only. For example, on the basis of observation alone, technical workers had disagreed as to whether or not terminal shoots grow longer in the "on" year than in the "off" year. The correlations obtained in these investigations have indicated quite definitely that they do grow longer in the "on" year. In some cases the results actually ran counter to prevailing opinion. For example, tree vigour was found to have little effect on the degree of biennial bearing. This was apparently due to the strong effects of meteorological conditions. Another surprising result was the lack of correlation between the organic matter content of the soil and tree yield.

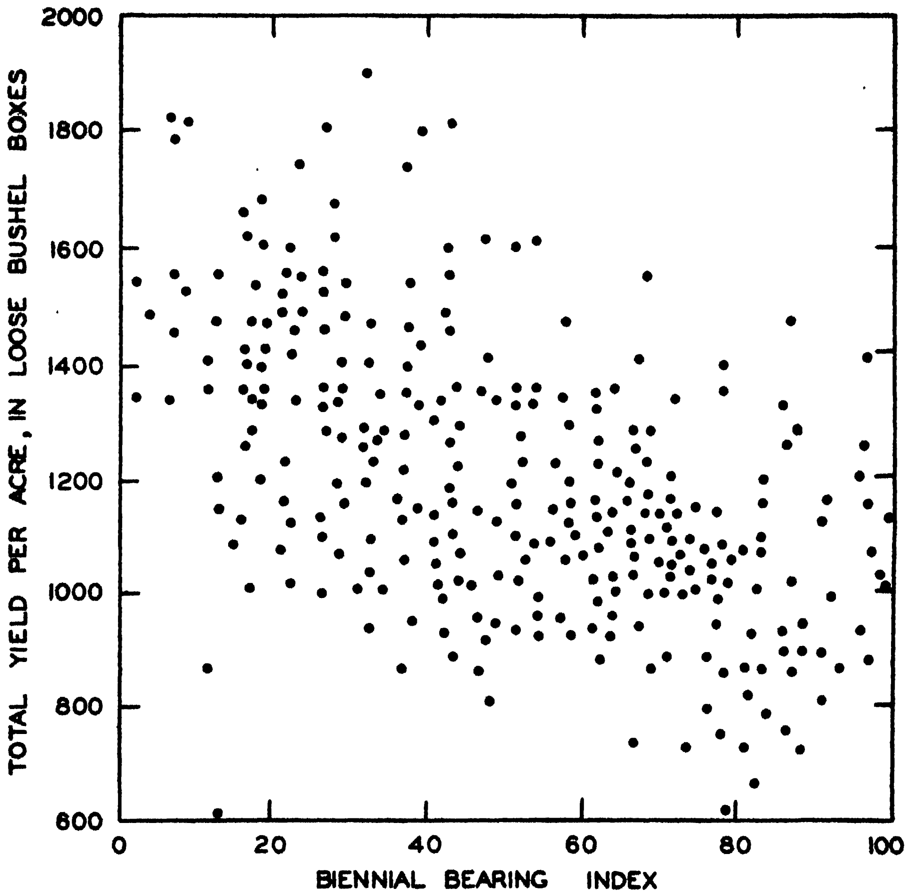

FIGURE 2. Scatter diagram of biennial bearing index charted against total yield per acre. r = − 0·526

Where there are so many factors affecting a function, it has been found very difficult to assess the proper relationships by observation alone. As an example, the correlation obtained between soil texture (as represented by the moisture-holding capacity) and tree yield may be cited. Many cases were found where it was obvious that trees in sandy soil were giving higher yields than trees in nearby heavy soils; yet the coefficients of correlation and the distribution charts showed clearly that on the average the reverse held true. This is illustrated in Figure 1, where moisture-holding capacity is charted against yield. Although in any one type of soil there was a wide range of tree yields, the general trend, for the average yield to increase as the soil became heavier, was apparent. Similar difficulties had been encountered in assessing the effects of biennial bearing on tree yield. As will be seen from Figure 2, large variations in yield occurred at any one degree of biennial bearing; yet there was a general trend for the yield to decrease as biennial bearing increased.

The large amount of valuable information obtained from these investigations indicates that the work was well worth-while.

SOME ADVANTAGES AND DISADVANTAGES OF THE SURVEY MOTHOD

Most of the main advantages and disadvantages of the survey method as encountered in these investigations have already been noted in the above discussion. It appears worth-while, however, to summarize them at this point.

Advantages

1. In comparison with the more exact procedures, such as growing plants in a greenhouse, the field methods have a distinct advantage in that they make use of field conditions. Field variability in soil type, climate, size and shape of tree could not well be duplicated in the greenhouse. It would of course not be feasible to study the factors affecting mature apple trees in the greenhouse. For example, it would not be feasible to study under glass the interaction of distance of planting and size of mature McIntosh trees. In some cases, it is doubtful if findings disclosed in the greenhouse could be applied directly to field conditions. One reason for this is the wide interaction of factors in the field compared with those in the greenhouse.

2. Using the survey method of research, it is not necessary to plan the experiment years ahead of time, plant the crop, and wait for it to reach maturity before obtaining the required data. This is of course of greatest importance with plants that take several years to reach maturity, as do fruit trees. Moreover, it is not necessary to set up a wide range of soil, nutritional or other conditions or treatments and wait for the differential effects to appear in plant behaviour. The fact is that the operator has at hand an outdoor laboratory, already set up for him in the grower-owned orchards or fields. The material is there, waiting for him to use it.

3. Even though a survey for the purpose of discovering relationships between factors and functions may not represent adequately the soils, plant materials or cultural operations over the area concerned, it should still provide good information about them. The operator gains a clearer picture of the status of the crop under consideration. Problems are discovered that show the need for further work; and suitable material is located on which to conduct this further work. Following the apple investigation, for example, it was possible to select orchards low in P and K on which to lay out fertilizer plots.

Disadvantages

1. The greatest disadvantage in the survey method is the multitude of factors with which the operator must contend. True, some of them can be eliminated and the range of others lessened in the selection of material; but there remains too large a group of factors for the operator to be able to analyse adequately all their individual effects and interactions. By the intelligent use of statistical procedures, it is usually possible to gain a clear picture of the major relationships involved. The minor relationships, however, may be so masked by the major ones that their discovery and interpretation are very difficult.

2. It is necessary to select more material for use in the investigational work than with plot work or greenhouse work. In order to obtain the

same records, therefore, it becomes necessary for the operator to spend more time at it. In addition, the time and expense involved are increased by the extra travel required.

3. The operator may find it difficult to control those factors receiving special study. For example, the grower may change his fertilizing, pruning, irrigating or spraying program during the process of the investigation. The operator of course has no control of the weather.

Purpose of a Research Survey

A research-type of survey can serve several good purposes. In the first place, it can reveal which factors are of greatest importance in affecting the functions, and which are of less importance. It can reveal those relationships existing among the major factors, and between these factors and the functions. It can reveal problems that need more exact study, and supply the materials with which to conduct further studies. To some extent it can be likened to a voyage of discovery prior to settlement of new lands.

The research type of survey cannot be expected to reveal exact relationships among the lesser factors, or between these factors and the functions. In order to establish effects of minor factors on yield or some other function, it is usually necessary to eliminate the effects of major factors in some way, such as can be done under strictly controlled experimental conditions. Nor can the survey method be expected to reveal information of a more purely fundamental nature, such as the function of an element in the plant. Although it can bring major relationships to light, it does not necessarily explain them. If any information of a strictly fundamental nature is obtained, it can be accepted by the investigator with pleased surprise.

The apple investigation did not indicate great promise for use of the survey method to determine the critical nutritional levels of soils or plants. Once standards of nutrient levels in the soil or plant tissues have been established, then the nutritional status throughout the area concerned can be determined by an ordinary survey involving soil and tissue analyses. In order to establish these levels in the first place, however, greenhouse, injection or field-plot studies hold greater promise than does the survey method. It is true that when a serious deficiency of an element occurs, a good correlation may be obtained between this element and plant performance; but usually other methods are better adapted to establishing this deficiency.

SUMMARY

Investigations of the survey type, previously reported by the author and his co-workers, are used as a basis for a discussion on the use of the survey method in horticultural research. Chief among these investigations were field studies of apple tree vigour, field and laboratory studies of soil moisture, and field and laboratory studies of factors affecting the yield of apple trees.

The major problem encountered in these studies was field variability, caused chiefly by the large number of factors concerned. Some of the factors were eliminated in part by selective choice of material, others by measurement and subsequent statistical treatment. Various procedures were used to glean from the data the various cause-and-effect relationships.

Difficulties were encountered, however, in interpreting the results obtained. In spite of all the difficulties encountered, the investigations were considered to be well worth-while.

The major advantages of the field survey method are: (1) that the results obtained are applicable to field conditions, (2) that the material is already waiting to be used, and (3) that the need for more exact work is revealed and the material necessary for this work is discovered. The chief disadvantages are: (1) that the multitude of factors makes interpretation difficult, (2) that on this account larger samples have to be measured and recorded, and (3) that it is difficult to maintain the factors at steady values during the course of the investigation.

It is concluded that the research-type of survey can serve several good purposes, such as revealing the major relationships between factors and functions. It cannot be expected to reveal minor relationships, nor relationships of a strictly fundamental nature. It does not show special promise for determining the critical values of nutrient elements in the soil or plant tissue.

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EXPERIMENTS ON THE SELF-SELECTION OF A DIET BY FOXES[1]

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INTRODUCTION

During experiments with dehydrated whole egg powder, at the Dominion Experimental Fox Ranch, it was noted that biotin-deficient foxes would consume brewers' dried yeast per se, which is a good source of biotin. It also has been observed that foxes housed in pens raised off the ground would eat fresh grass when this was provided, even though they were being fed an apparently well balanced diet. These facts suggested that the fox instinctively sought to balance its own ration when provided with the necessary foodstuffs. "Self-selection" or "free choice" experiments were inaugurated in order to study this more completely.

REVIEW OF THE LITERATURE

One of the earliest reports on self-selection feeding was by Evvard (6) who worked with pigs and concluded that the appetite of the pig was a reliable indication of bodily needs. However, Nevens (9) found that cows do not select feeds in the proper amounts or the proper proportions to meet their nutrient requirements. Osborne and Mendel (10) provided rats with two diets, one nutritionally superior to the other. When confronted with these two experimental diets, the rats showed a definite preference for the superior diet.

A number of workers (1, 3, 11, 12, 15, 17, 18, 27) have reported abnormal food preferences in both laboratory animals and humans, under abnormal anatomical or physiological conditions, which indicated that the species under observation was trying to satisfy a nutritional need. Richter and co-workers (13, 16) found that rats have the ability to select a balanced ration during growth, pregnancy, and lactation. Scott and co-workers (22) observed a variation in preference for certain nutrients between old and young rats and between pregnant and non-pregnant rats. They also noted (19) that, aside from salts and calories, the appetites of rats showed no apparent relation to physiologic or nutritional needs, and that rats relish a change in the carbohydrate portion of their ration (23).

Kon (8) reports that rats on a self-selection diet consumed a ration that contained only 6.5 per cent protein. These rats did not grow as well as the control animals receiving a mixed ration containing 20 per cent protein. Carlson and Hoelzel (2), using a vegetarian self-selection diet, found that rats were generally fertile on such a diet but raised less than 25 per cent of their offspring. The growth of the progeny also was subnormal and severe rickets commonly developed. Some workers in this field have not provided the animals with all of the required nutrients. The vitamin aspect particularly has been neglected. For instance Dove

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(5) provided his rats with cornmeal, wheat bran, oats, skimmilk powder, fishmeal, bonemeal and oyster shell, which undoubtedly would be low in vitamins A and D.

Deuell and Movitt (4) found that rats prefer butter to hydrogenated vegetable oils, while Scott and Verney (25) report the reverse to be true, which would indicate that rats, as a species, do not have clear-cut likes and dislikes in their feeding habits.

No reports, to the authors' knowledge, have appeared in the literature regarding the ability of the fox to select an adequate diet.

PROCEDURE

Two separate experiments were carried out, using slightly different procedures. In the first experiment 28 feeds that are commonly found in fox rations were used, while in the second experiment the number of feeds was limited to seven. These seven feeds contained all of the essential food nutrients known to be required by foxes.

Experiment No. 1

Three trials were carried out in this experiment. In the first trial, six 8-week-old weanling pups were used (three males—7B, 8B, and 18B, and three females—1B, 2B, and 3B). In the second trial, six 20-week-old or half-grown pups, which were the same animals as used in the first trial, were placed on the self-selection diet. In the third trial, four adult foxes (7A, 45A, 131A, and 65Z) were used.

The following 28 feeds were placed before the foxes in individual pans: alfalfa meal, cooked barley, raw barley, dried beet pulp, blood meal, commercial steamed bonemeal, fortified cod liver oil (1800A-800D), dehydrated whole egg powder, fresh frozen fish (Novascot Fishblox), fishmeal, ground flaxseed, fresh grass clippings, ground cabbage (when grass was out of season), ground horsemeat, ground limestone, linseed meal, ground beef liver, meat meal, molasses, cooked oats, raw oats, iodized salt, skimmilk powder, ground beef tripe, wheat bran, wheat germ, cooked wheat, raw wheat and brewers' dried yeast.

In preliminary trials it was found that foxes preferred cereal grains in the dry, rather than in the moist, state and that they showed a preference for grains ground through a 1/16 inch screen rather than a 1/8 inch or 1/32 inch screen. It also was observed that grass clippings were eaten more readily than ground grass. Using the findings from these preliminary trials, the barley, oats, wheat, and beet pulp were ground through a 1/16 inch screen and were fed dry. The cooked grains were autoclaved for 15 minutes at 15 lb. pressure. The feed pans were placed in wall brackets about eight inches off the floor in a large room. The room was so situated that the foxes could be observed without the observer being seen. Each day the foxes were placed in the room for approximately 30 minutes or until they finished eating. The fox pups were weighed once a week. The feed consumed was recorded each day and at the end of each week the percentage composition of the ration was calculated in order to note any changes in food preference as growth and the season progressed.

TABLE 1.—WEEKLY PERCENTAGE COMPOSITION OF RATIONS CONSUMED BY FOXES ON SELF-SELECTION RATIONS

	Pups						Half-grown pups				Adults			
	1st week	2nd week	3rd week	4th week	5th week	6th week	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week
Alfalfa meal	—	—	—	—	0.01	—	—	—	—	—	—	—	—	—
Barley, cooked	—	0.01	0.03	—	0.01	—	—	0.01	0.01	0.03	0.02	—	—	—
Barley, raw	—	0.03	0.01	0.02	0.03	—	0.01	0.01	0.01	0.02	0.05	0.02	0.02	—
Beet pulp	0.01	0.03	0.03	0.01	—	—	—	—	—	—	—	—	0.02	—
Blood meal	0.01	0.01	0.03	0.01	0.04	0.04	0.01	—	—	—	0.02	—	—	—
Bonemeal	0.10	0.05	0.08	—	0.01	0.01	0.03	—	0.01	—	—	—	—	—
Cod liver oil	0.71	0.04	0.21	0.09	0.03	0.01	0.02	0.06	0.14	0.01	0.91	0.04	0.11	0.09
Egg powder, whole	—	0.07	0.01	0.02	0.07	0.10	0.03	0.01	0.04	—	0.07	—	0.09	0.06
Fish, fresh (fishblox)	0.19	0.05	0.16	0.54	0.09	0.12	0.05	0.11	0.04	0.12	0.29	—	0.24	0.09
Fishmeal	0.02	0.02	0.02	0.01	0.03	—	0.01	0.02	0.01	0.02	0.02	0.05	—	—
Flaxseed, ground	0.04	—	—	0.02	0.01	—	—	—	—	—	0.02	—	—	—
Grass, fresh	0.02	0.09	0.13	0.13	0.08	0.07	0.04	0.11	0.07	0.02	0.10*	—	—	—
Horsemeat	49.28	85.90	80.35	71.37	77.70	76.20	74.50	80.50	90.75	88.92	80.30	78.94	69.10	67.00
Limestone, ground	—	0.07	0.24	0.11	0.11	0.12	0.02	0.01	0.01	0.04	0.05	—	—	—
Linseed, meal	—	0.01	0.02	0.01	0.01	—	0.01	0.01	—	—	—	—	—	—
Liver, beef	17.76	8.01	13.20	24.60	17.70	20.15	16.20	12.30	5.60	7.49	4.36	6.54	5.95	0.47
Meatmeal	—	0.07	0.06	0.04	0.04	0.04	0.03	0.02	—	0.03	0.02	—	—	0.03
Molasses	—	—	0.06	0.04	0.08	0.07	0.55	0.38	0.39	0.06	2.01	1.22	0.29	0.22
Oats, cooked	—	0.02	0.02	—	0.02	—	0.01	0.03	0.01	—	0.02	—	—	—
Oats, raw	—	0.01	0.02	0.01	0.02	0.01	0.01	0.04	0.01	0.02	—	—	0.02	—
Salt, iodized	0.04	0.02	0.03	0.01	0.04	—	0.01	0.01	—	0.01	—	0.02	—	—
Skimmilk powder	0.17	0.07	0.13	0.09	0.04	0.02	0.09	0.02	—	—	0.05	0.05	0.04	—
Tripe, beef	31.57	5.31	5.00	2.85	3.70	3.02	8.28	6.30	2.85	3.14	11.60	13.10	24.12	31.95
Wheat bran	—	—	0.02	0.01	0.02	—	0.01	0.01	—	0.01	0.05	0.02	—	—
Wheat germ	—	0.03	0.07	—	0.02	—	0.01	0.01	0.03	0.04	0.02	—	—	0.09
Wheat, cooked	—	0.04	0.02	—	0.02	0.01	0.02	0.02	0.01	—	0.02	—	—	—
Wheat, raw	—	0.01	0.01	—	0.03	—	0.01	0.01	0.01	0.01	—	—	—	—
Yeast, brewers' dried	0.08	0.03	0.04	0.01	0.04	0.01	0.04	—	—	0.01	—	—	—	—

* Ground cabbage fed in place of fresh grass in adult feeding trial.

Experiment No. 2

Harris *et al.* (7) found that when a large number of feeds were placed before rats, they became confused and rarely selected the nutrients that would produce the most beneficial results. In order to determine if this was the reason why the foxes in the first experiment consistently had chosen an unbalanced ration, six pups were placed on a self-selection diet containing the following seven feeds:

(1) Casein as a source of protein.
(2) Ground beef suet as a source of fat.
(3) Ground beet pulp as a source of fibre.
(4) Fortified cod liver oil for vitamins A and D.
(5) Brewers' dried yeast for the vitamin B complex.
(6) Minerals (4 bonemeal: 1 salt).
(7) Dehydrated potatoes as a source of carbohydrates.

The procedure, other than the number of feeds used, was identical with that followed in the first experiment and the pups were maintained on this ration for three weeks.

RESULTS AND DISCUSSION

Experiment No. 1

(1) At the end of five weeks on the self-selection diet the weanling fox pups 1B and 7B were showing symptoms of rickets and one week later the hind legs of 7B were almost useless. During this trial the six foxes had consumed a ration containing only 0.18 per cent cod liver oil as is shown in Table 1, instead of the 1½ to 3 per cent recommended by some workers. They also showed a definite preference for ground limestone rather than steamed bonemeal. Fresh meat (horsemeat, liver, and tripe) made up 98.9 per cent of the total feed consumption during the six-week period. The average daily feed consumption per fox for the six weeks was 242 grams of feed while normal consumption for a growing pup is 300 grams and more.

(2) The six foxes from the first trial were placed on a cod liver oil supplemented ration and two weeks later 1B and 7B were again apparently quite normal. After six weeks on this ration the same pups, now approximately 20 weeks of age (half-grown), were again placed on the self-selection diet. At the end of four weeks 7B again showed definite symptoms of rickets and as none of the other foxes was consuming what was considered a balanced ration the experiment was discontinued. It is significant that 7B, the first fox to become rachitic, grew more rapidly than any other fox in the experiment as is shown in Table 2. In fact the growth curve was almost normal for a fox of that age. The cod liver oil consumed during this period was 0.06 per cent of the total ration. The fresh meat (horsemeat, liver, and tripe) constituted 99.2 per cent of the total ration consumed and the average daily consumption per fox was 274 grams of feed for the four-week period.

(3) The four adult foxes which were placed on the self-selection diet persisted in consuming a ration which was considered to be nutritionally unbalanced, so the trial was discontinued at the end of four weeks. The ration consumed during the four-week period averaged 98.4 per cent fresh

meat (horsemeat, liver, and tripe) and the average daily consumption per fox was 222 grams of feed. Normally, adult foxes consume about 280 grams of feed per day. It is of interest to note that the adult foxes consumed a higher per cent of tripe but a lower per cent of horsemeat and liver than the pup foxes in the first trials.

TABLE 2.—WEEKLY BODY WEIGHTS OF FOX PUPS ON SELF-SELECTION DIETS

Age in weeks	Fox numbers					
	1B lb.-oz.	2B lb.-oz.	3B lb.-oz.	7B lb.-oz.	8B lb.-oz.	18B lb.-oz.
8	4–00	4–01	4–04	3–08	3–10	3–10
9	3–14	3–12	4–01	3–12	3–12	3–14
10	4–00	4–02	4–05	4–01	4–02	4–04
11	4–14	4–12	5–04	5–02	5–00	4–03
12	5–03	4–15	5–08	5–06	5–06	4–09
13	5–04	5–06	6–00	5–10	5–08	4–14
14	5–00	5–11	6–04	5–14	5–12	5–10
20	7–14	8–01	9–03	9–15	8–08	8–08
21	7–02	7–14	8–12	10–08	8–12	8–10
22	7–08	8–06	9–04	11–00	9–05	9–00
23	7–12	8–06	9–08	11–04	9–12	9–04
24	7–08	8–08	9–06	11–08	9–14	9–06

Experiment No. 2

At the end of three weeks the pups on the self-selection diet, containing only seven feeds, had lost an average of 10 ounces in body weight which was at a period in their life when they normally would be gaining 8 to 10 ounces a week. Because of the severe losses in body weight the experiment was discontinued at the end of the three-week period. The average daily consumption of feed for this three weeks was only 20 grams. The percentage composition of the ration consumed was as follows:

	Per cent
Suet	96.50
Cod liver oil	2.72
Brewers' yeast	0.36
Beet pulp	0.18
Minerals	0.14
Casein	0.05
Potatoes	0.05

It will be noted that the pups, during this test, consumed considerably more cod liver oil than was consumed by the pups in Experiment 1.

SUMMARY AND CONCLUSIONS

Six weanling pups, six half-grown pups, and four adult foxes were placed on a self-selection diet with 28 feeds from which to choose. None of the groups selected a nutritionally balanced diet since the fresh meat made up 98.8 per cent of the ration consumed in all groups. In both pup groups a rachitic condition developed even though cod liver oil, bonemeal, and limestone were available to them. The results indicate that neither weanling pups, half-grown pups, nor adult foxes are able to select, from this large assortment of feeds, those which are essential to their growth and physiological well-being.

In a second experiment six pups were placed on a self-selection diet in which there were only seven feeds from which to choose. The results of this experiment showed that, even when fox pups have only a limited number of feeds from which to choose, they still are unable to select a nutritionally balanced ration.

ACKNOWLEDGMENTS

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PERSIAN DARNEL IN CANADA[1]

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During the past two decades an annual weed, locally known as "darnel", has become well established at numerous widely-separated points in the Prairie Provinces. While not yet abundant enough to cause serious economic losses to grain producers, it indeed may come to do so in the near future. It seems appropriate to put on record now some information of a botanical nature that has recently been gathered about the grass.

HISTORY OF NOMENCLATURE

Much confusion has existed in Canada as to the correct botanical name of this plant. Being an annual and resembling somewhat the well-known darnel of Europe, it was first called *Lolium temulentum* L. A specimen from wheat fields in the vicinity of Saskatoon sent to A. S. Hitchcock in Washington in 1925 by W. P. Fraser was identified more particularly as *L. temulentum* var. *macrochaeton* A. Br., the variety with long awns usually considered typical of that species. From subsequent correspondence and from additional specimens received at Ottawa from W. P. Fraser, it was apparent that this identification was not wholly acceptable to him. Since the exact identity remained in question, this varietal combination continued in use (6).

With the appearance of Rydberg's "Flora" in 1932 and the reference therein to an awned variety of *Lolium rigidum* as "the only form represented, with an awn 7-12 mm. long. Wheat fields: N.D., introduced from the Old World", the new combination name of *L. rigidum* Gaud., var. *Duethiei* Hook. was soon taken up for the weed introduced to the grain fields in Saskatchewan. Soon afterwards, Hitchcock's "Manual of the Grasses of the United States" (8) referred simply to *L. rigidum* as "annual, foliage blue-green, spikes rather stiff; otherwise like *L. multiflorum*—Wheat fields, North Dakota (Milton, Calio); Europe". Both locations in North Dakota mentioned by Hitchcock are within 30 miles of the Canadian border and since the brief description seemed sufficient for general reference, the bare specific name became widely used in agricultural literature—despite the fact that the plants are not particularly blue-green when fresh and the spikes not particularly stiff, at least not nearly so stiff and rigid as in the plants that Gaudin had in mind when he proposed the name *L. rigidum*. Dr. Malte, in annotating a specimen, pointed out that Gaudin's original description (5) stated that the spikelets were awnless, but left it at that. Although Professor Fraser used *L. rigidum* in his Saskatchewan list (4) he was apparently not fully satisfied with it, since he appended, "exact species has not been determined".

IDENTIFICATION OF DARNEL

In attempting to arrive at an acceptable identification of the darnel, it was apparent that recourse to original descriptions and authentic specimens had to be taken. There was no doubt that the grass was a species of

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Lolium. The sessile spikelets, one at each node, inserted dorsally on the rachis with the first glume entirely lacking (except in the terminal spikelet), places it distinctly within this genus.

Lolium is not a native American genus. Most of the species in the New World were introduced from Europe in times of early settlement. A search of European floras, however, yields no description exactly applicable to the weed now passing as *L. rigidum* in North America. Some of its distinctions from the European species with which it has been confused may be mentioned. It differs from *L. temulentum* var. *macrochaeton* in its more slender stature, lanceolate (rather than elliptical) lemmas, in glumes shorter (rather than longer) than their spikelets, and palets longer (rather than shorter) than their lemmas. From *L. temulentum* var. *leptochaeton* it differs, in addition, by the presence of awns. Both varieties of *L. temulentum* have been introduced to Canada on numerous occasions but seem not to have become naturalized. It differs from the true *L. rigidum* of Gaudin in its more slender rachis, its firmer and more coriaceous lemmas and presence of awns. *L. rigidum* has recently been introduced into Australia and, under the trade name of Wimmera Rye-grass has gained considerable importance as a forage species. By some workers there, it is rated as the most useful and most palatable grass for range pastures in the drier and often saline areas in Australia (3, 12). The slender, almost leafless and worthless weed of our western grain fields could not possibly pass under the same scientific name without creating much suspicion. *L. multiflorum* is a short-lived perennial with short glumes and herbaceous lemmas, frequent in new lawn and meadow seedings, and readily distinguishable from our darnel.

The awned variety of *L. rigidum*, cited by Rydberg (11) as var. *Duethiei* Hook., is an Asiatic variety, first described by Hackel in J. D. Hooker's, "Flora of British India" (9) in 1896. In this work it bears the original spelling of var. *Duthiei* and authorship correctly ascribed to Hackel. The description was based on specimens collected by J. F. Duthie in Kashmir, now preserved in the herbarium of the Royal Botanic Garden, Kew, England. A duplicate specimen of Duthie's type in the United States National Herbarium has been examined and found to be distinctly different from Canadian darnel. Specimens from Milton, North Dakota, which probably provide the basis for Rydberg's and Hitchcock's reports of *L. rigidum*, likewise do not compare well with the type material. It is concluded that *L. rigidum* or its variety *Duthiei* does not occur in North America. Details of the spikes of the species mentioned in the above two paragraphs are shown in Plate I.

IDENTITY AND DESCRIPTION OF *Lolium persicum*

On consultation of modern Russian floras (7, 10), the Canadian plants key out directly to *L. persicum* Boiss. & Hoh., a species ranging from the Caucasus through northern Iran, to the mountainous parts of Turkestan, Syr-Darya, Pamir, and Tien Shan in middle Asia. The type specimen was collected in the district of Derbent, Persia (Iran), by T. Kotschy in 1843 and is preserved in the herbarium at Geneva, Switzerland.

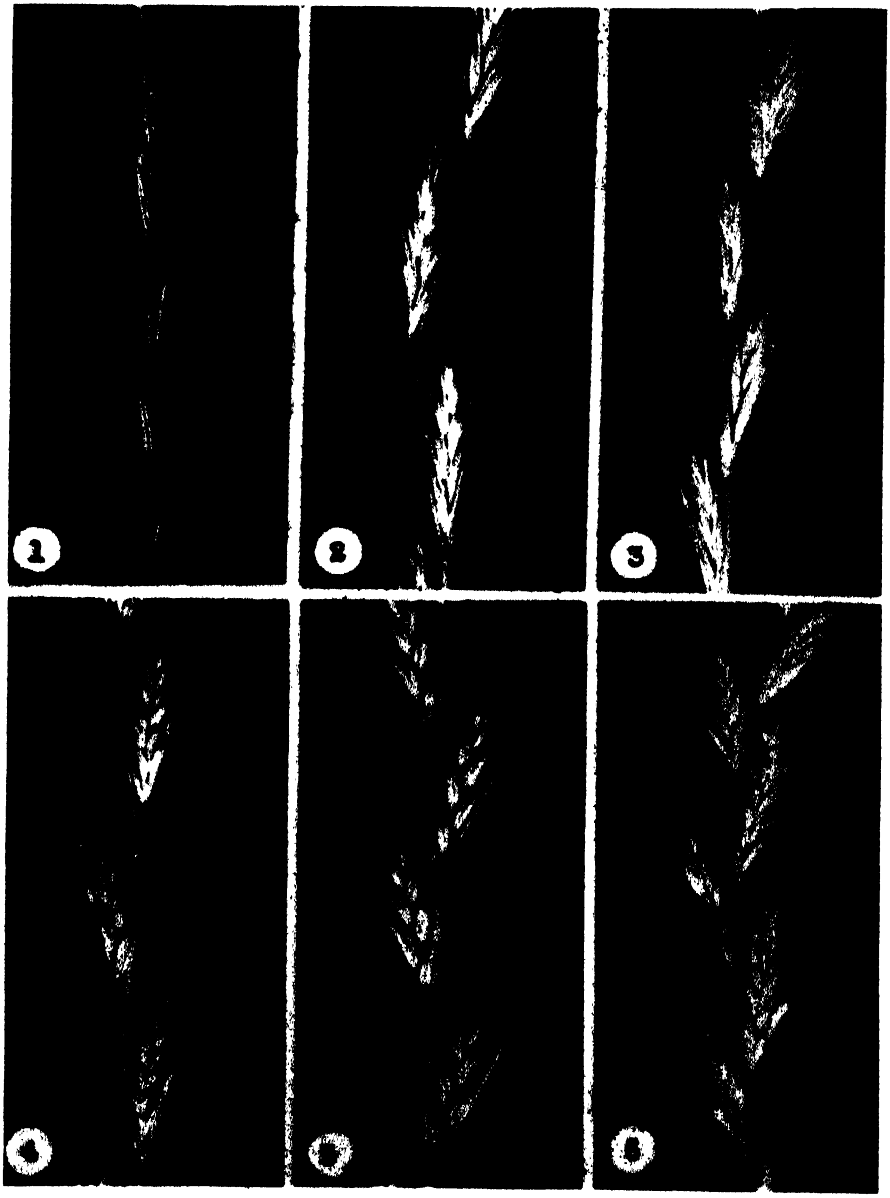

PLATE 1. Spikes of annual species of *Lolium*. FIGURE 1. *L. rigidum* var. *Duthiei* from specimen of type material from Kashmir, preserved in U.S. National Herbarium. FIGURE 2. *L. persicum* from Russia, cultivated at Forage Division, Experimental Farm, Ottawa. FIGURE 3. *L. persicum* from Simpson, Saskatchewan. FIGURE 4. *L. temulentum* var. *macrochaeton*. FIGURE 5. *L. temulentum* var. *leptochaeton*. FIGURE 6. *L. multiflorum*. FIGURES 4, 5, and 6 from plants cultivated at Forage Division, Experimental Farm, Ottawa. All spikes magnified 1.6 times.

The original description by Boissier (2) is inadequate in some details, but it is clear that Nevski (10), when expanding on the original description, had in mind the same species as Boissier, and undoubtedly referred to the co-type specimen preserved at Leningrad. His full description, and also other specimens collected in the type locality in 1902 by J. Bornmüller, labelled by the collector as *L. persicum* and preserved at Washington, agree perfectly with Canadian and North Dakota material. There remains no doubt that the plants introduced to North America are of this species. Spikelets of this species from Russia and Canada are shown in Plate I (Figures 2 and 3).

The distinctive common name Persian darnel may be proposed for general use.

The following description based on Canadian material is designed to bring out the main diagnostic points:

Lolium persicum Boissier & Hohenhaker. Pl. Pers. bor. 1846. *L. rigidum* of American authors, not Gaudin.

Bright green *annual*, blue-green on drying, 15 to 60 cm. tall, freely branching from the lower nodes, the branch stems closely ascending and all terminating in inflorescences. Blades 2 to 6 mm. broad, rough on the margins and nerves of the upper surface, smooth beneath. Spike one-quarter to one-half the height of the stem, with *slender rachis*. Spikelets 5- to 7-flowered, 1.5 to 2.2 cm. long (exclusive of awns). *Glume three-quarters to full length of spikelet*, 1.0 to 1.8 cm. long, diverging from rachis at angle of 20° to 30°, indistinctly 5- to 7-nerved. Lemmas coriaceous, the lower one in the spikelet five times as long as broad, about 10 mm. (8 to 11 mm.) long and 1.5 to 2.0 mm. broad (not spread out), *with slender awn of a length* (0.8 to 1.2 cm. long) *equal to that of body of the lemma* and arising just below its tip. Palet equal to, but *generally surpassing its lemma in length* by 1 to 2 mm., with straight firm and hispid-ciliate keels gradually converging into a slender point and *exposed along the upper half of the lemma*. Caryopsis 6 mm. long, 1.5 mm. broad.

In Canada two forms are represented. In one form, the culms are coarsely scabrous beneath the spike; in the other, the culms are smooth. The former is typical of the species; the latter, however, represents an undescribed form. In the citations below these two forms will be listed separately and in chronological order of collection.

CITATION OF SPECIMENS

In the following list, herbaria are designated by the following abbreviations: A—University of Alberta, Edmonton; Can—National Herbarium of Canada, Ottawa; DAO—Division of Botany, Department of Agriculture, Ottawa; DAS—Laboratory of Plant Pathology, Dominion Department of Agriculture, Saskatoon; DASC—Range Experiment Station, Dominion Department of Agriculture, Swift Current, Sask.; DAScott—Experiment Station, Dominion Department of Agriculture, Scott, Sask.; DAW—Rust Research Laboratory, Dominion Department of Agriculture, Winnipeg, Man.; M—University of Manitoba, Winnipeg; OAC—Ontario Agricultural College, Guelph; S—University of Saskatchewan, Saskatoon; US—United States National Herbarium, Washington.

I. Specimens of the rough-culmed form typical of the species:

Ontario:

Mortimer Island, north shore of Lake Superior, Thunder Bay District, old camp clearing, *Hosie, Losee & Bannan 1298*, July 27, 1937 (Can.). Nipigon, railway gravel, *Groh 77*, July 10, 1939 (DAO). Guelph, three specimens, all probably from same infestation in yard of Veterinary College and collected by students; *L. M. Marshall*, July 12, 1946 (OAC), *S. J. W. Rickord*, July 16, 1946 (DAO), and *W. M. Stacey*, July, 1946 (OAC).

Manitoba:

Benito, *Murray*, August, 1934 (DAO). Winnipeg, grass garden, Manitoba Agricultural College, *T. Johnson*, July 10, 1935 (DAW). St. Norbert, roadside, *Groh*, July 9, 1935 (DAO). Domain, patches in grass field, *E. Johnson*, June 27, 1941 (DAO). Makaroff, *Van Drecht*, July, 1948 (M).

Saskatchewan:

Saskatoon, *Fraser*, June 17, 1923 (DAS). Saskatoon common in wheat fields, *Fraser*, August 13, 1925 (US). Langham, *Russell & Scott*, July 6, 1926 (DAS). Saskatoon, plots, *Fraser*, July, 1926 (S). Tisdale, *Laister*, August, 1926 (DAO). Saskatoon, weed in grain fields, *Fraser*, July 11, 1928 (DASC). Sutherland, *Fraser*, 1929 (S). Simpson, *Walker*, fall 1931 (DAO). Thirteen miles west of Simpson, *Groh & Walker*, June 21, 1932 (DAO). Langham, *Groh*, June 23, 1932 (DAO). Carnduff, *Groh*, June 27, 1932 (DAO). Hafford, wheat fields, *Shevkenek*, June 24, 1933 (DAScott). McKague, cultivated field, *Breitung*, July 6, 1936 (S). Swift Current, experimental plots, *Budd*, July 13, 1936 (DASC). Radville, low area, *Heinrichs*, July 4, 1938 (DASC). Senlac, cultivated grain fields, *Gould per Russell* S1399, June 23, 1944 (DAO).

Alberta:

Altario, grain fields, *Black*, received March, 1936 (DAO). McLaughlin, submitted by *Carpenter*, July 16, 1936 (A). Edmonton, R. R. track, C.N.R. yards, *Sexsmith & Moss 4297*, June 23, 1937 (A). Spirit River, 2 miles west in roadside ditch, *Moss 7492*, July 23, 1947 (DAO, A). Spirit River, one mile north in roadside ditch and field, *Moss 7493* (DAO, A). Spirit River, station yard, *Groh 3069a*, September 7, 1946 (DAO). Spirit River, *Shewchuk*, July 10, 1947 (DAO). Codesa, trace infestation believed introduced in 1947, *Shewchuk*, July 20, 1948 (DAO). Northmark, quite bad infestation believed introduced in 1943, *Shewchuk*, July 22, 1948 (DAO).

United States:

Fargo, North Dakota, grown from seeds received from Milton, N.D., *Stevens*, July 25, 1911 (US). Fargo, grown from seeds found in wheat from Milton, *Stevens*, July 7, 1919 (US). Fargo, grown in garden, seed from Cavalier Co., 1941, *Stevens 623*, July 13, 1942 (DAO). Madison, Wisconsin, grown in weed garden, *Stone*, August, 1922 (US).

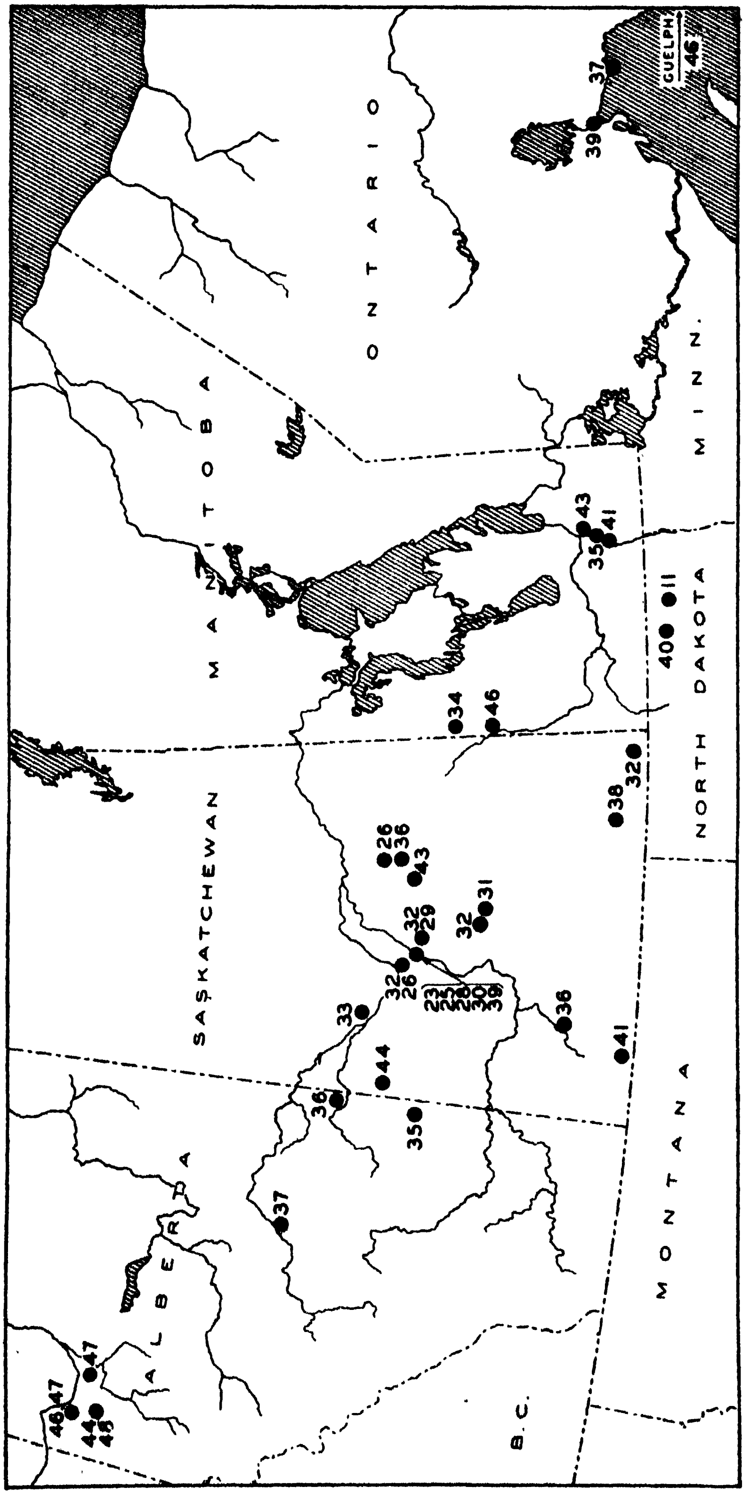

PLATE 2. Locations and dates of collection of the 38 specimens of *Lolium persicum* preserved in herbaria.

II. Specimens of the smooth-culmed form:

Manitoba:

North Kildonan, Winnipeg, farm, *Denike, 1789*, July, 1943 (DAO).

Saskatchewan:

Sutherland, *Fraser*, 1929 (S). Saskatoon, wheat fields, *Russell*, June 27, 1930 (DAS). Saskatoon, cultivated field, *Russell*, July 26, 1939 (DAO, DAS). Val Marie, irrigated field, *Campbell*, Aug. 1, 1941 (DASC). St. Gregor, cultivated field, *B. J. S. & Russell*, July 20, 1943 (DAS).

Alberta:

Northmark, Peace River, grain fields, *Rowe*, July, 1944 (DAO).

OCCURRENCE AND SPREAD

The present distribution as indicated by the herbarium specimens listed above is shown in the map (Plate 2). The figures by each dot refer to the year in the present century when the collections were made.

From these specimens, the history of the invasion of *L. persicum* reads somewhat as follows: The weed became established in Cavalier County in northeastern North Dakota some time prior to 1910; in that year, seeds were taken from grain samples and grown by Stevens at Fargo for identification purposes. The plants were reported as *L. temulentum* by Bergman in 1912 in his flora of the state (1). Stevens (13) reported these plants and others cultured from the same source in 1919 under the name of *L. rigidum* var. *Duthiei*. By 1930 it was considered a serious enough weed to be included in a revision of the North Dakota weed bulletin (14). In 1919 extensive distribution of durum wheat took place from Cavalier County.

The first specimen collected in Canada came from Saskatoon, where Professor Fraser detected it in 1923. In 1925 he noted it as "common in wheat fields, Saskatoon". In 1926 it was found at Langham and Tisdale, west and east of Saskatoon. In the "thirties" it appeared at several other points in central Saskatchewan and had spread as far as Carnduff and Radville in the southeastern corner, Swift Current in the southwest, and eastward across the border to Benito, and St. Norbert (near Winnipeg), Manitoba. The spread also occurred westward into Alberta, first near the border at Altario and McLaughlin, then at Edmonton. In 1937 and 1939, respectively, it had reached Mortimer Island and Nipigon on the north shore of Lake Superior in Ontario.

The weed got into the Peace River District of Alberta about 1943 and as far east as Guelph in 1946 (see below). C. E. Hubbard, grass specialist at the Royal Botanic Garden, Kew, who kindly verified the identity of Canadian specimens, writes that "only last year (1947) it turned up on refuse heaps around flour mills at Tewkesbury, Gloucestershire, England, where the occurrence of an Asiatic species was hard to explain since most of the grain coming into the country for milling came from Canada".

The present status of the weed may be gathered from information provided by agricultural workers in their respective areas. In North Dakota, it grows in various parts of the State, including the western counties (O. A. Stevens). In central Saskatchewan, Persian darnel is now regarded as an established weed. Some experiments have been conducted to study its germinability and control. It is quite common some ten to fifteen miles south of Scott, where it is a serious pest in the Revenue area. It is plentiful on light land and covers a fairly extensive acreage south of North Battleford (H. A. Friesen).

In the Peace River block of Alberta, the weed appears to be spread generally in the area around Spirit River and extends as far as Wanham and Sexsmith. It is inconspicuous in initial infestations but later tends to become so thick that it chokes out the grain. Since Persian darnel grows about 10 to 12 inches high, much of it remains uncut in the stubble and some seed escapes from the butts of the sheaves. In addition, it is difficult to separate from grain in the ordinary farm fanning mill (E. C. Stacey). It is interesting to note that the specimen collected at Northmark in 1944 by Rowe is the smooth-culmed form, while those collected from the "quite bad" infestation there in 1948 by Shewchuk are of the commoner rough-culmed form. This seems to indicate that the weed is being spread by mass means as a heavy impurity in seed grain or feed.

In August, 1948, a rapid search was made for the weed in three regions near Winnipeg—at St. Norbert, Domain, and North Kildonan, where it had been collected earlier. No plants, however, were located. If the weed had gained prominence in that region, it would have been brought more to the attention of agricultural authorities. The infestation at Makaroff in eastern Manitoba, thought to be introduced in seed grain in 1945, however, was still active in 1948 occurring in patches over about five acres of grain land. The weed is not widely recognized in that area (C. van Drecht), but its occurrence indicates that other outbreaks are to be expected in Manitoba.

The plants from Mortimer Island, a rugged island of the Slate Group about 10 miles off Schreiber along the Lake Superior shore, were undoubtedly introduced with feed-stuffs during lumbering activities. The full collections of the University of Toronto botanists show numerous weed introductions to these islands. At Guelph, agricultural students located plants (probably escaped from western feed) at the rear of the Veterinary College in July, 1946. The infestations at the Mortimer Island and Guelph sites may not be expected to persist, due, in the former case, to competition from natural vegetation and, in the latter, to competition from students eager to collect classroom material.

SUMMARY

The annual weedy grass which, due to the inadequacies of our floristic manuals, has been passing as *Lolium temulentum* var. *macrochaeton*, *L. rigidum*, *L. rigidum* var. *Duthiei*, or *L. multiflorum* is identified as *L. persicum* Boiss. and Hoh., a species native to south-western Asia. Some of its distinctive characters are the slender rachis, firm lemma with slender awn of equal length, glume equal or slightly shorter than the spikelet, and the sharp-pointed palet which exceeds the lemma in length. Most of the specimens are rough on the culm, but some smooth-culmed plants, which belong to an undescribed form, are also present in Canada.

Persian Darnel first appeared in North America in northeastern North Dakota about 1910 and spread subsequently to various parts of that State. The first Canadian specimens came from Saskatoon in 1923. Since then it has spread westward to the Peace River area of Alberta and eastward into Manitoba. In 1946 it was detected as far east as Guelph, Ontario, and in 1947 was reported from refuse heaps around flour mills at Tewkesbury, England, where Canadian grain was being milled. It is a weed of grain fields in the Prairie Provinces and is apparently being spread in commercial seed and feed grain.

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A SOIL RATING AND CLASSIFICATION FOR IRRIGATION LANDS IN WESTERN CANADA[1]

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The development of extensive irrigation projects in western North America during the first quarter of this century brought into focus many problems hitherto unrecognized. One of them was the realization that irrigated lands varied in their ability to produce and, therefore, in their ability to carry the capital cost of construction. In 1923 the United States government appointed a fact-finding commission to study this and related problems. In submitting its report to Congress in 1924, President Coolidge said in part: "The heretofore adopted repayment plan is erroneous in principle. It fixes an annual arbitrary amount that the farmers must pay . . . regardless of their production. In its place should be substituted a new policy providing that payments shall be assessed . . . in accordance with the crop producing quality of the soil." This need, of assessing the land on the basis of the productive power of the soil, became evident in the Lethbridge Northern Irrigation Project in Alberta early in its history. The first water was applied in 1924 and in 1929 a soil survey was requested of the irrigated lands of this project (1). This survey was under the direction of the late F. A. Wyatt of the Department of Soils of the University of Alberta. A "Table of Ratings", including a soil rating table, was compiled by the Irrigation Council of Alberta and was presented to the Government of Alberta in February, 1930. This soil productivity rating was the one used to rate the soils of the Lethbridge Northern Project. Subsequent to this, the soils on other irrigation projects in southern Alberta were classified and rated (2).

In 1936 the Saskatchewan Soil Survey, at the request of the Water Development Committee of the P.F.R.A., undertook to make soil surveys and to rate the soils in proposed irrigation projects. The following year the Alberta Soil Survey began similar work. Moss (3) outlined the objectives of this type of survey in a paper given at Lethbridge in June, 1939. Since 1936 these two Surveys have classified, mapped and rated in proposed irrigation projects, approximately 400,000 acres by detailed survey and 2,500,000 acres by reconnaissance survey (4).

In the development of a soil rating system specific to irrigable lands on the Canadian Prairies, the same general principles were used as in the rating of land for dry land agriculture, but the emphasis given to the various physical factors was often quite different. As a simple example: In the semi-arid brown zone the heavy textured soils are given top rating for dry land agriculture, whereas for irrigation purposes they rate much lower than do the soils of medium texture.

The ratings reported in this paper are those currently used by the Saskatchewan and Alberta Soil Surveys and are an outgrowth and modification of the original "Table of Ratings" referred to earlier. In 1943 the late J. Mutchler, then senior survey engineer for P.F.R.A., requested that the two surveys prepare a uniform classification and rating for irrigation soils. As a result of this request, a meeting was held in Lethbridge, Alberta, in August, 1943, to review the existing data, and to unify the classification systems used by the two Surveys*. The report of this meeting has been the basis for future work (5). Some modifications have been made in individual ratings when necessary but basically the system as reported in this paper is that decided upon at Lethbridge in 1943.

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[1] Presented to the Soils Section of the Agricultural Institute of Canada at Vancouver, B.C., June, 1949.

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The method described in this paper for classifying soils for irrigation can be used as a fairly specific guide. However, it is not yet possible to evolve a system whereby the effects of all soil factors can be accurately tabulated, related, and applied to obtain a definite mathematical rating. The personal judgment of trained and experienced pedologists is still required in any system of soil rating.

In classifying and rating soils for irrigation the following factors must be considered:

1. *Soil Profile*—The type of soil as shown by the morphology and chemical composition of the profile.
2. *Type of Geological Deposition* of the parent material with special reference to its uniformity.
3. *Soil Texture*—of both surface and subsoil. In relation to the subsoil, consideration must be given to a depth of at least five feet.
4. *Salinity*—The amount, kind and position of the salts in the profile.
5. *Degree of stoniness.*
6. *Wind and water erosion*—if any.
7. *Topography* and its associated drainage pattern.

In rating any soil it is imperative that all the above factors receive recognition and also that no one factor gets recognition in more than one place.

1. *The Soil Profile*

The rating of this factor applies primarily to profiles of the brown and dark brown soil zones since it is considered that most of the irrigation development has been and will be in these zones. The type of soil profile has a bearing on the vertical movement of water through the soil and also on the conditions affecting plant growth. Only the more extensive profile types are listed in this paper; other types will and do occur.

(a) The regional or normal profile—namely, the brown earth and chestnut earth profile. *Rating 100.*

(b) Alluvium. (Immature profiles of river flood plains). *Rating 100.*

(c) The solonchak (saline) profile. Structurally this profile *rates 100.* Deductions, however, must be made for the salt content and this is taken care of under the section on "Salinity".

(d) Solonetzic or solodic profile. These are soils that show some development of the heavy compact B horizon. *Rating 80-90.*

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* The following attended this meeting: J. Mitchell, University of Saskatchewan; J. D. Newton, University of Alberta; J. L. Doughty, Dominion Soil Laboratory, Swift Current; W. E. Bowser and H. C. Moss, Dominion Experimental Farms Service. E. S. Hopkins, Associate Director, Dominion Experimental Farms Service, attended the first session. F. A. Wyatt was unable to attend but assisted in subsequent discussions carried on by correspondence.

(e) Solodized solonetz, the hard columnar-pan profile. Shallow and deep variations of this profile occur as well as varying degrees of hardness of the B horizon. Eroded pits are often associated with this soil type. *Rating from 60-80.*

(f) Calcareous earth profile. This soil has a weakly developed B horizon that is usually calcareous. *Rating 90.*

(g) Very calcareous profile. This soil has a limy A horizon directly over the B_{ca} horizon. The rating given depends on the amount of carbonate and its closeness to the surface. *Rating 70-90.*

2. *Type of Geological Deposition*

The kind of geological deposition of the parent material in so far as it influences the movement of water through this material affects the value of soil for irrigation purposes. This factor is concerned primarily with the lateral movement of water and the consequent development of seepage (vertical movement is considered under "Profile Types" and "Soil Texture").

The following main types of surface deposits are encountered:

(a) Glacial lacustrine deposits, medium to heavy textured, uniform throughout their depth. *Rating 100.*

(b) Alluvial-lacustrine and aeolian deposits, light to medium textured, uniform throughout. *Rating 100.*

(c) Glacial till—somewhat variable. There is very little information on the movement of irrigation water through glacial till primarily because few glacial areas have been irrigated. Since the material in these deposits is usually quite variable, and also as the configuration of the particles in a till deposit may be quite different to the configuration in an alluvial or a lacustrine deposit, it is difficult to postulate whether appreciable lateral movement of water will take place or not. It is expected that some seepage will occur and that the somewhat irregular topography of the till areas will facilitate seepage. It is suggested that the ratings be based upon the amount of variability and on the degree of compaction. *Tentative rating 60-80*—subject to revision when the behaviour of till under irrigation is better known.

(d) Alluvial deposits—variable throughout depth (that is, they contain lenses of widely different textures as well as gravel). *Rating 50-80.*

(e) Sorted or modified residual deposits. The depth to consolidated bedrock and the nature of the bedrock must be considered. *Rating 20 to 100* (that is, the 20 rating would apply to soils with less than 1 foot of profile over the consolidated bedrock).

(f) Glacial outwash; gravelly and stony. *Rating 20 to 50.*

3. *The Soil Texture*

The soil texture is a very important factor in irrigation. The ratings given below assume the "normal" texture gradient for the profile type; that is, in most profiles a gradual increase in texture weight with depth.

Where an abrupt textural difference occurs which is not taken care of in the parent material or profile factor, it must be considered here and will modify the rating given the surface texture.

There have been considerable changes in kind of crops grown and in cropping practices over the years and this has necessitated changes in the texture rating. In general this has meant a raising of the rating for some of the light and medium textures. The following rates apply to the more frequently occurring textures:

Silt loam	100
Loam	90–100
Silty clay loam	90
Clay loam	90
Very fine sandy loam	90
Sandy clay loam	90
Fine sandy loam	80–85
Silty clay	75–85
Sandy loam	70–75
Clay	50–70
Coarse sandy loam	60–65
Loamy sand	50–60
Highly dispersed clay	30–50
Sand	30–50
Gravel	20

4. *Salinity*

It is very difficult to accurately forecast the amount and extent of salt (alkali) damage that may develop with the application of irrigation water. The amount of salt present in the soil and the uniformity or lack of uniformity of the parent material are the principal determiners of future damage. Some general estimation, however, can be made. The relative uniformity of the parent material is given recognition in Section 2 above. The present section deals with the amount and kind of salt present. In general it is considered that soils containing over 1 per cent of water-soluble "white alkali" salts in the upper two feet (exclusive of calcium sulphate) are unsuitable for irrigation. Small areas of soil with this high a salt content, lying within large areas of desirable land, will quite possibly be irrigated and may produce some fodder; however, it would be unwise to recommend the irrigation of large tracts of this type.

In rating the salinity factor, consideration must be given to the texture, the profile features and the general fertility of the soil, as these may modify the final rating given.

For "black alkali", concentrations of sodium carbonate of 0·1 per cent or over are regarded as too high for satisfactory crop growth. Even slight amounts of "black alkali" reduce the productive value of the soil. In general any soil with a pH value above about 8.8 in the upper two feet is considered dangerous.

RATING FOR WHITE ALKALI

"None" to "traces"	Rating 100
0·2 to 0·4 per cent	Rating 75–95
0·4 to 0·7 per cent	Rating 50–85
0·7 to 1·0 per cent	Rating 25–60
Over 1·0 per cent	Rating 20–30

The highest ratings apply to the heavier textured soils, the lowest rating to the light textured soils. These ratings apply to the upper 24 inches of soil. High concentrations below this depth are a potential source of "alkali" trouble, and such a condition should be covered in the report and some deductions made for this in the rating. In soils high in sulphate and in which soluble sodium and magnesium are considerably higher than calcium, the limits reported in this table must be reduced considerably.

5. *Degree of Stoniness*

The degree of stoniness affects the cultivation of the land rather than its irrigability. It is a factor, however, that must be considered. The ratings used are quite similar to those used by the soil survey for dry land agriculture rating.

No stones	Rating 100
Few stones	Rating 85–95
Moderately stony	Rating 60–85
Very stony	Rating 30–60
Excessively stony	Rating 20

6. *Erosion*

Loss of top soil by erosion reduces the value of the land and must receive consideration. Considerable judgment must be used in the application of this factor since a given loss in one soil type may not be of equal value to loss in another soil type.

The rating given varies from 100 for relatively no loss, to about 60 if all the A horizon is removed. The patchy removal of the A horizon in the solodized profile is considered under this factor and is rated on the basis of percentage loss to the base figure of 60.

Very severe erosion, such as deep wind-eroded pits and water-cut gullies, must receive separate consideration.

7. *Topography*

The final topographic classification must be made from plane table sheets to determine exact irrigable acreage, size of field, mileage of canal and percentage slope. However, for proposed projects it is generally necessary for the soil surveyor to make a preliminary topography classification. The classification and ratings listed below were compiled by checking topography classes against plane table sheets and lumping all the deductions made for size of field, percentage slope etc. into one topographic factor. In general, this factor is a rating of the irrigable portion of the parcel, and is only indirectly affected by the amount of non-irrigable land.

	Rating
1. *Level to very gently sloping* 0·2 per cent to 2 per cent uniform slope—all irrigable......	90–100
2. *Very gently undulating* Irregular hummocky surface that requires levelling. With levelling most of area irrigable—0·2 per cent to 2 per cent slope..	70–90

3. *Gently sloping*

   Frequency of 1 or less roll per half mile. All irrigable on assumption that water will reach highest point............ 70–90

4. *Gently undulating*

   Up to three rolls per half mile; slopes may be in more than one direction. Some non-irrigable land—2 per cent to 5 per cent slope.. 60–75

5. *Roughly undulating*

   Four or more rolls or knolls per half mile. Considerable non-irrigable land—2 per cent to 5 per cent slope.............. 20–55

6. *Flat and depressional land*

   Usually less than 0·1 per cent slope. Its value will depend largely on the feasibility of constructing drains............ 20–50

7. *Gently rolling*

   Irregular surface, and moderately sloping regular surface. Considerably non-irrigable to all non-irrigable............. 20–50

8. *Rolling, hilly and steeply sloping*

   This includes areas that are rough, broken and eroded...... 15–35

When the above seven factors are individually rated, these ratings are all multiplied together to give the final rating. The final ratings are then fitted into the groups given in Table 1. This grouping is then superimposed on the basic soil map or is reported on a separate map.

TABLE 1.—CLASSIFICATION TABLE FOR RATING SOILS FOR THE PURPOSE OF IRRIGATION

Group	Description	Rating	Map colour
1	Very good soils	100–73	Blue
2	Good soils	72–53	Green
3	Fair soils	52–40	Yellow
4	Poor or doubtful soils, or soils of limited use	40–25	Orange
5	Unsuitable	Below 25	Red

It is noted that there is an increase in category range with each higher class. There is a range of 27 points in Class I land, 19 points in Class II and 12 points in Class III. It is possible to subdivide, if required, at least the two top classes.

The following example illustrates the use of this rating system. Chin light silt loam is a regional brown earth profile of alluvial-lacustrine deposition. It is the dominant profile in the St. Mary's development project. An average Chin light silt loam rates about 100 for profile, 100 for texture, 90 for topography, 90 for salinity and 90 to 95 for the deposition factor. Multiplying these together gives a final rating of about 75; that is the lower limit of Class I. Any additional detrimental variation, as for example in topography, would place an area down into Class II.

It is known that certain changes may occur within the soil profile as the result of irrigation. For example, in the original levelling of the micro-relief certain changes in the surface texture occur. Wyatt, in a paper presented to the Western Society of Agronomy in June 1939, lists the following changes:

(1) Change in soil volume
(2) Change in soil structure
(3) Change in fertility
(4) A translocation and possible concentration of the water soluble salts.

This last-mentioned change is also reported by Marshall and Palmer (6). These factors cannot be fully assessed prior to irrigation and some reclassification of problem areas may be necessary subsequent to irrigation.

ACKNOWLEDGMENTS

The authors wish to emphasize that the information presented in this paper is a contribution of the Alberta and Saskatchewan Soil Surveys, and that all those who have been associated with irrigation soil surveys in the two provinces during the past twenty years have contributed in some measure to this subject.

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EFFECT OF INADEQUATE BROOD SOW RATIONS ON THE PRENATAL AND POSTNATAL DEVELOPMENT OF THE PROGENY[1]

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INTRODUCTION

Interest in brood sow nutrition has been stimulated by the results of a number of studies (1, 2, 6, 7, 8, 9) recently conducted in the United States which have shown that rations, formulated to contain adequate protein, minerals, vitamins A and D, and energy for growth, may be strikingly inadequate for the support of normal reproduction and lactation in brood gilts and sows maintained continuously in dry lot. These, and other studies, emphasize the importance of earlier observations regarding the value of pasture and alfalfa hay in brood sow nutrition, and demonstrate that rations which are satisfactory for pigs being grown and fattened for market may be deficient in nutrients required for normal gestation and lactation.

Although reproduction and lactation failures of the severity of those reported by American observers are not normally encountered by Canadian swine producers who make proper use of the available grains and supplements, serious losses do occur in herds in which sows are maintained under conditions approaching continuous dry lot feeding on rations containing very limited amounts of protein, mineral or vitamin supplements.

The present report outlines the results of a series of experiments initiated in the summer of 1944 to obtain data on the performance of an experimental group of sows maintained over an extended period in dry lot on a ration composed simply of oats, barley, ground limestone and salt, as contrasted to that of a comparable control group fed oats and barley supplemented with a protein-mineral mixture, feeding oil and pasture in season. Representative gilts from both groups of sows, after being grown out in dry lot to a weight of from 200 to 225 lb. on a ration of oats and barley supplemented with a protein-mineral mixture, but not with feeding oil, were maintained on the same ration as that fed their respective dams. Data are presented to show that the performance of the second generation females fed the experimental deficient ration after reaching breeding age was inferior to that of their dams as measured by numbers of pigs farrowed and weaned, and that their productivity was not fully restored by subsequent supplementation with either alfalfa meal or mixed protein-mineral supplement, feeding oil and pasture.

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[1] This study was conducted with the aid of a grant from Burns and Company, Limited, Calgary, Alta.

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[4] Part of the data in this paper were taken from a thesis submitted by H. H. Draper in partial fulfilment of the requirements for the degree of Master of Science.

EXPERIMENTAL

SECTION I. REPRODUCTION STUDIES

The experiments subsequently described are divided into three series, A, B, and C, according to the nature of the experimental ration. In Series A, comprised of five farrowing seasons, the ration for the experimental group contained grains and minerals. In the Series B experiments, covering three farrowing seasons, the ration of grains and minerals was supplemented with alfalfa meal. During Series C, consisting of two farrowing seasons, all sows, including those previously deprived of supplementary protein and pasture, were maintained on the control ration which included protein supplement, minerals, feeding oil and pasture in season. With one exception, noted below, the experimental rations were fed continuously.

Series A Experiments

Object

To obtain data on the long-term performance of sows deprived of pasture and greenfeed and fed a ration considered deficient in protein, vitamins A and D, and possibly other factors, as compared to that of sows fed a good quality ration including pasture during the summer.

Procedure

Experiment 200—Summer 1944

Two groups of four two-year-old purebred Yorkshire sows of similar breeding were used in this experiment. They and their dams had previously been maintained on a ration made up of coarse grains, mixed protein-mineral supplement, and either pasture or alfalfa hay plus fish oil.

With the exception of a five-month period (see Experiment 200B) the four original sows in each group were fed the rations shown in Table 1 from June 1944 to November 1946. As indicated below, a number of daughters of the original sows were placed on the same feeding regime as their dams after they had reached breeding age.

TABLE 1.—RATIONS—SERIES A EXPERIMENTS

	Group I—Control		Group II—Experimental
	On pasture	In dry lot	
	%	%	%
Ground Oats	47	44	49
Ground Barley	47	44	49
Mixed Supplement*	6	12	—
Feeding Oil**	—	½ oz. per sow per day	—
Iodized Salt	—	—	1
Ground Limestone	—	—	1

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* The mixed supplement was composed of: Tankage 50, linseed oil meal 25, alfalfa meal 15, ground limestone 5, and iodized salt 5 pounds per 100 pounds of mixture.

** The feeding oil was guaranteed to contain 1500 I.U. of vitamin A and 200 I.U. of vitamin D per gram.

The crude protein content of the rations of both groups varied considerably with different sources of coarse grains, but analyses indicated that on an air-dry basis the protein content of the dry lot ration for the control group ranged from approximately 14.0 to 15.0 per cent, while that of the experimental group varied from 10.5 to 11.5 per cent.

Group I received cereal or rape pasture during the summer. The experimental sows of Group II were subjected to continuous dry lot feeding in vegetation-free yards. Sows of both groups were moved into the piggery for farrowing and the first three or four weeks of lactation.

All young pigs were treated with three weekly doses of reduced iron and 3 c.c. of feeding oil containing 1500 I.U. of vitamin A and 200 I.U. of vitamin D per gram.

Experiment 200B—Winter 1944–45

Owing to a critical labor shortage it was necessary to maintain both groups of sows on a gestation ration consisting of oats 60, barley 34 and mixed protein-mineral supplement 6 per cent. At farrowing they were returned to their respective rations as outlined for Experiment 200.

Experiment 208—Summer 1945

As for Experiment 200.

Experiment 208B—Winter 1945–46

Three gilts were selected from the second litters of each group and added to their respective lots. The procedure of Experiment 200 was continued.

Experiment 215—Summer 1946

Two additional second-generation gilts were included in each group, and a number of the original sows were discarded so that only one of these remained in Group I and two in Group II.

Results and Discussion

The farrowing and weaning records of the experimental and control groups for the five experiments of Series A are summarized in Table 2.

Despite the absence of protein supplement, feeding oil and pasture from the ration fed the experimental group no serious breeding difficulties were encountered during these experiments. As indicated in Table 2, the control sows farrowed an average of 2.7 more live pigs than those of the experimental group, and by weaning the disparity in litter size had increased to 3.6 pigs. While the control sows weaned 74 per cent of their pigs and the experimental sows only 55 per cent, the progeny of the latter group that survived to weaning age were as heavy at that time as those of the former. That the vigour of the pigs from the test sows at weaning was impaired, however, was indicated by post-weaning losses of 22 per cent for the experimental group and 13 per cent for the controls.

In spite of the continuous absence of fish oil in the experimental ration, no gross manifestations of prenatal vitamin A deficiency, such as malformed or stillborn pigs, were observed. To what extent the weakness of many of the progeny of the test sows and their susceptibility to infection were attributable to a deficiency of this vitamin is speculative.

TABLE 2.—FARROWING AND WEANING RECORDS—CONTROL VS. EXPERIMENTAL SOWS, SERIES A

	Number sows bred	Number sows farrowed	Total number pigs born		Average number born alive*	Average birth weight, lb.	Number weaned		Per cent weaned	Age at weaning, days	Average weaning weight, lb.
			Alive	Dead			Total	Mean*			
Experiment 200											
Control	4	3	38	1	12.7	2.8	28	9.3	74	56	26.7
Experimental	4	4	40	1	10.0	2.5	24	6.0	60	56	23.1
Experiment 200B											
Control	4	4	48	2	12.0	2.5	41	10.3	85	59	27.9
Experimental	4	3	27	2	9.0	2.7	17	5.7	63	54	23.6
Experiment 208											
Control	4	3	33	2	11.0	2.5	19	6.3	58	55	23.1
Experimental	4	4	38	0	9.5	2.6	11	2.8	29	56	21.4
Experiment 208B											
Original sows:											
Control	4	4	41	3	10.2	2.4	27	6.7	66	48	25.1
Experimental	4	4	37	0	9.2	2.8	21	5.2	57	59	31.7
Daughters:											
Control	3	3	20	1	6.7	2.6	15	5.0	75	57	27.7
Experimental	3	3	15	4	5.0	2.6	13	4.3	87	60	28.5
Experiment 215											
Original sows:											
Control	1	1	14	0	14.0	2.0	12	12.0	86	67	24.4
Experimental	2	2	19	1	9.5	3.0	14	7.0	74	61	33.7
Daughters:											
Control	5	4	48	0	12.0	2.5	38	9.5	79	62	24.3
Experimental	4	4	24	2	6.0	2.7	10	2.5	42	66	23.7
Totals and Means											
Control	25	22	242	9	11.0	2.5	180	8.2	74	57	25.9
Experimental	25	24	200	10	8.3	2.7	110	4.6	55	58	26.7

* Basis no. sows farrowed.

First vs. Second Generation

A comparison of the farrowing and weaning records of the first and second generation sows, control and experimental, is shown in Table 3.

TABLE 3—FARROWING AND WEANING RECORD—FIRST VS. SECOND GENERATION, SERIES A

Group	Generation	Number litters farrowed	Number pigs born alive		Number weaned		Per cent weaned
			Total	Mean	Total	Mean	
I—Control	First	15	174	11.6	127	8.5	73
II—Experimental	First	17	161	9.5	87	5.1	54
Difference in favour of Group I		−2	13	2.1	40	3.4	19
I—Control	Second	7	68	9.7	53	7.6	78
II—Experimental	Second	7	39	5.6	23	3.3	59
Difference in favour of Group I		0	29	4.1	30	4.3	19

The effect of the experimental regime on the second generation may be gauged by comparing the performance of the first and second generations of the test sows with that of the controls. The difference in litter size of 1.9 pigs between generations of control sows is assumed to be due to the normal decrease in the size of first litters. The corresponding difference of 3.9 pigs for the experimental sows shows an additional decrease of 2.0 pigs per litter which may be charged against the effect of the test ration. The disastrous consequences of the experimental regime are indicated by the fact that the second generation test sows weaned an average of only 3.3 pigs per litter.

Series B Experiments

The data presented above for the experiments included in Series A show that continuous feeding of a ration of grain, ground limestone and iodized salt to the brood sows in the experimental group led to a marked decline in the number of pigs weaned by the sows of this group. The series B experiments, covering a period of three farrowing seasons, were conducted to determine whether the addition of alfalfa meal to the ration of these sows would lead to any improvement. The rations fed to Group II during this period were as follows:

	First 6 months	Succeeding 12 months
	%	%
Oats	43	41
Barley	43	41
Alfalfa meal	12	16
Ground limestone	1	1
Iodized salt	1	1

The alfalfa meal used was of medium quality and contained an average of 14 per cent protein on an air-dry basis. The protein content of the complete ration, as estimated from intermittent analyses, ranged from 11 to 12 per cent.

The sows and gilts used were daughters of sows in Group II of Series A. Four out of nine of these daughters had farrowed either once or twice while they were being fed the original Group II ration of grain, ground limestone and salt. The remaining five farrowed for the first time after being fed this ration supplemented with alfalfa meal during pregnancy. The control sows of group I were also the daughters of the original group I—Series A animals, and they were continued on the feeding regime followed in this group during the Series A tests.

Results

The average results for the three farrowing seasons included in the period of 18 months during which the ration of group II was supplemented with alfalfa meal are summarized in Table 4.

TABLE 4.—EFFECT OF ALFALFA MEAL SUPPLEMENTATION

	Group I (control)	Group II (alfalfa meal)
Number litters possible	20	18
Number litters born	18	16
Per litter:		
Average number pigs born alive	11.8	9.9
Average number pigs born dead	0.6	0.5
Average number pigs weaned	7.9	6.1
Per cent pigs weaned	67	61
Average weaning weight, lb.	24.6	19.7
Average number pigs marketed	7.0	4.9
Per cent pigs marketed	61	50

Comparison of the averages listed for Group II in Tables 4 and 2 for numbers of pigs born and weaned per litter shows that 1.6 more pigs were farrowed and 1.5 more were weaned by the sows in the Series B experiments than by those in the Series A experiments. These results, obtained following the addition of alfalfa meal to a ration of cereal grains, salt and ground limestone, suggest that alfalfa meal supplementation may bring about some improvement in production performance even with sows or daughters of sows that have been fed an obviously inadequate ration over an extended period. However, from a comparison of the results for Groups I and II in Table 4 it is apparent that supplementation simply with alfalfa meal failed to raise the production performance of the sows in Group II to a normal level. Pigs from these sows averaged 19.7 lb. in weight at weaning and post-weaning death losses were high, so that only 4.9 pigs out of each litter reached market weight.

The chief beneficial effect of alfalfa meal supplementation, under the conditions of these experiments, appeared to be an increase in the number of pigs born per litter. A greater number of pigs were weaned per litter by the sows in group II of the Series B experiments than by those in the same group in the Series A trials, but this increase in litter size at weaning was, to a large extent, offset by the fact that the average weight at weaning was 7.0 lb. less per pig.

Series C Experiments

These experiments were conducted to determine the improvement in reproductive performance that might result from feeding a more complete ration, including pasture in season, to sows selected from group II of Series B. Sows from Group I of Series B were used as controls. Both groups were fed the same ration, viz. that used for Group I in the Series A and B experiments. Records are complete for two farrowing seasons—the fall of 1948 after the sows of both groups had been on cereal and rape pastures for approximately four months, and the spring of 1949. Farrowing, weaning and marketing records for the two seasons are summarized in Table 5.

TABLE 5.—EFFECT OF FEEDING CONTROL RATION TO SOWS OF GROUP II OF SERIES B

	Group I	Group II
Number litters possible	13	11
Number litters born	10	8
Per litter:		
Average number pigs born alive	11.5	11.1
Average number pigs born dead	0.5	0.0
Average number pigs weaned	8.9	8.2
Per cent pigs weaned	77	74
Average weaning weight, lb.	26.9	21.8
Average number pigs marketed	8.5	7.2
Per cent pigs marketed	74	65

The trend toward better performance in Group II during the Series C experiments was similar to that obtained in the Series B trials. That is, there was a further increase in litter size to an average of 11.1 pigs as compared to 11.5 farrowed by the sows of Group I. Improvement is apparent also in the percentage of pigs weaned in Group II—74 per cent during the Series C trials as compared to 61 per cent for Series B and 55 per cent for Series A. The average weaning weights remained low at 21.8 lb. and post weaning death losses in Group II were 9 per cent as compared to 3 per cent in Group I. These results, obtained with sows characterized by a previous history of poor nutrition, suggest that the effects of feeding inadequate rations to brood sows over an extended period may be at least semi-permanent in nature.

SECTION II. PROGENY FEEDING TRIALS

Object

To augment the reproduction data which have been presented in Section I, five feeding trials (Nos. 208, 208B, 215, 218 and 221) were conducted to ascertain the effect of the ration fed to the sows during the Series A and B experiments upon the growing and fattening performance of their progeny.

Procedure

Representative weanling pigs from each group of sows were allotted as uniformly as possible with respect to sex, weight, dam, condition and type, to lots of from five to seven pigs each. The pigs were housed in a piggery without access to soil or sunlight. Straw was used for bedding. In some instances the numbers of test pigs available did not permit as high a degree of uniformity in allotment as might have been desirable, but continued repetition of the trials resulted in the establishment of averages which are believed to constitute a fair basis for comparison of the growth and fattening performances of progeny of the control and experimental sows. Weights were recorded at the beginning of the experiment and every fourteen days thereafter until the pigs reached a market weight of approximately 200 pounds.

Each feeding trial was divided into two periods: Period I, from weaning to 110 lb.; and Period II, from 110 lb. to market weight. The basal rations fed are listed below:

	Period I	Period II
	%	%
Ground oats	35	10
Ground barley	53	84
Mixed supplement*	12	6

* The mixed supplement was composed of: Tankage 40, linseed oil meal 35, fish meal 15, ground limestone 5, and iodized salt 5 pounds per 100 pounds of mixture.

The average protein contents of the above rations on an air-dry basis were 14.4 per cent for the first period and 12.4 per cent for the second. It will be noted that the rations for both periods might be regarded as deficient in vitamins A and D. For purposes of comparison, the two lots of Experiment 221 and the first and third lots of Experiment 218 (see Table 6), control and experimental, were given fish oil at the rate of 4 c.c. per pig per day.

Results and Discussion

A summary of the results of the five feeding trials is presented in Table 6.

Although the differences in the feed lot performance between the pigs from the control and test sows were small, they were relatively consistent. According to Student's *t* test for unpaired values, the average differences of 0.09 lb. per day in daily gain and 19.9 lb. feed consumed per 100 lb. gain are highly significant ($t = 19.83$ and 3.67 respectively).

Occasional instances of staggering and muscular incoordination were observed among the pigs from sows of both groups. Pigs that exhibited only mild symptoms of incoordination were left untreated and in most cases made a gradual recovery without any serious loss of appetite or decrease in rate of growth. The most severe cases were treated individually with feeding oil. Recovery was rapid following treatment and the attacks were attributed to vitamin A deficiency. No cases of incoordination were observed in the groups that received feeding oil in the ration.

TABLE 6.—RESULTS OF FEEDING TRIALS—PROGENY OF CONTROL AND EXPERIMENTAL SOWS

Group	Lot No.	Number of pigs	Average initial weight	Average final weight	Average daily gain	Average feed per 100 lb. gain
			lb.	lb.	lb.	lb.
Experiment 208						
Control	1	6	29.2	195.3	1.23	369.0
Experimental	1	6	29.3	193.3	1.05	400.5
Experiment 208B						
Control	1	7	42.4	202.6	1.43	378.2
Experimental	1	5	36.3	201.4	1.31	397.9
Control	2	9	26.1	194.6	1.29	369.2
Experimental	2	6	24.2	194.7	1.24	381.6
Experiment 215						
Control	1	7	35.3	200.4	1.12	378.6
Experimental	1	5	34.0	194.6	1.05	416.1
Control	2	5	29.8	199.2	1.16	391.6
Experimental	2	6	27.0	201.2	1.11	365.0
Experiment 218						
Control	1*	7	24.4	194.3	1.07	367.4
Experimental	1*	5	21.0	189.6	1.04	381.1
Control	2	6	34.3	209.2	1.20	375.2
Experimental	2	7	31.1	194.3	1.01	420.0
Control	3*	7	39.4	201.1	1.16	380.2
Experimental	3*	5	42.2	202.2	1.22	408.6
Experiment 221						
Control	1*	5	45.4	200.8	1.37	377.9
Experimental	1*	5	24.8	195.6	1.20	387.9
Totals and Averages						
Control		59	33.6	199.4	1.22	375.7
Experimental		50	29.8	196.2	1.13	395.6

* Feeding oil containing 1500 I.U. vitamin A and 200 I.U. vitamin D per gram was fed to these lots at the rate of 4 c.c. per pig per day.

From the data accumulated during the reproduction and feeding trial studies, it appears that the effect of the deficient ration fed to the experimental sows was exemplified primarily in their reproduction and weaning performance rather than in the growing-fattening record of the progeny. Growing pigs which survive beyond the weaning stage and are subsequently maintained on a ration of cereal grains supplemented with protein and minerals are evidently capable of performing with moderate success, even though the ration of their dams was of poor quality.

GENERAL DISCUSSION

Considering that the ration fed to the sows in Group II during the Series A experiments was not supplemented with protein or feeding oil, the results obtained were in some respects unexpectedly favourable. Evidently no factors were deficient to such a degree that the ability of the sows to conceive was affected. The incidence of abnormalities at birth was zero, and the number of pigs born dead was the same for the two groups. In accordance with the relative litter size, the average birth weight of pigs from sows of the experimental group was 0.2 lb. greater than that of those from the control sows. No explanation is apparent for the fact that, despite a continued absence of a vitamin A supplement in the test ration, no gross evidence of a prenatal deficiency of this vitamin was observed in the progeny of the sows in Group II. Secondary infections, particularly those of the respiratory tract, were common during the suckling and post-weaning stages.

Gwatkin (3) and Gwatkin and Plummer (4, 5) have reported on the effect of continuously feeding a ration of grain, salt and ground limestone to brood sows and their offspring. Although data are reported for only a small number of sows, they showed a high incidence of breeding difficulties, of stillborn pigs and of deaths before weaning. Progressive depletion in succeeding generations was also demonstrated, the losses prior to weaning in the first to third generations being 26, 60 and 100 per cent respectively.

In the present experiments the main impact of the deficient regime was registered during the lactation period. Many of the pigs from the test sows became thin, emaciated and diarrhoeic within a few days after birth, and by weaning age the incidence of mortality reached 45 per cent. Of the remainder, 22 per cent died during the post-weaning period, leaving a group whose performance during the growing and finishing stage compared relatively favorably with that of the control pigs.

It is difficult to state accurately to what extent a deficiency of milk production existed on the part of the test sows, but it is doubtful if there was a shortage during early lactation. On the other hand, the milk supply of some of the sows became meagre during the later stages of the nursing period.

An attempt was made to assess the financial significance of the losses incurred in these experiments through the continuous use of the deficient sow rations fed to Group II in the Series A and B trials. Gross revenues from the sale of pigs marketed from the sows of groups I and II were calculated from current prices. Deductions were made for feed, labour, boar service, sow replacement, interest and depreciation on investment in buildings, etc., and, in the case of Group I, for pasture and fencing. The calculated net return from group II over a period of 3½ years was only one-quarter that from group I.

SUMMARY

Experiments were conducted to determine the effect of maintaining an experimental group of sows continuously on a ration consisting of small grains, iodized salt and ground limestone, as compared to the effect of maintaining a control group on a common practical ration which included coarse grains, mixed protein-mineral supplement, feeding oil, and pasture in season.

The breeding efficiency of the experimental sows as measured by their ability to conceive was not impaired by the deficient ration; but their prolificacy, as compared to that of the control group, was reduced by 2.7 live pigs per litter. No abnormal or stillborn pigs were observed. Mortality among the progeny of the test sows was high during the lactation and post-weaning periods. The deleterious effect of the experimental regime was more pronounced in the record of second generation sows than in that of the first. In terms of average daily gains and feed consumption per 100 lb. gain, the feeding performance of the progeny of the control sows was somewhat superior to that of the progeny of those in the experimental group.

The addition of alfalfa meal to the ration fed to the sows of the experimental group caused an increase in average litter size and number weaned, but the vigour and weaning weights of the progeny were not improved. When the control ration, including pasture in season, was fed to the depleted sows of the experimental group, a further increase was obtained in the numbers of pigs born and weaned per litter, but the average weaning weights remained low.

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ON THE EFFECT OF CRUDE BENZENE HEXACHLORIDE ON CEREAL SEEDLINGS

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There are numerous published references to the unfortunate effects of benzene hexachloride on the development of plants, both from seed (11, 13, 18) and from the tuber (1). In only one of these reports (18), however, is any reference made to the question of the exact identity of the constituent of the crude material which is responsible for these effects. Crude BHC as marketed contains about 70 per cent of the alpha isomer, about 5 per cent of the beta isomer, 12 per cent gamma isomer, some delta isomer, and small amounts of other materials. Of these, the only constituent recognized as important as an insecticide is the gamma isomer. Although from the supposed resemblance in spatial configuration of the molecules of gamma benzene hexachloride and iso-inositol (2, 10, 16) it seemed likely that this isomer was also responsible for the effects on plants, this theory of the activity of gamma BHC now appears to be disproved (3, 20). If the effects on plants were due to some other constituent which could be readily extracted from the crude material, the potentialities of this material for the control of wireworms and other soil insects, both by the preferred method of seed treatment and otherwise, would be greatly enhanced. To settle this question, experiments were begun in March, 1948, by treating seed wheat with various benzene hexachloride preparations and observing the effects on germination and development.

EXPERIMENTS WITH BENZENE HEXACHLORIDE FORMULATIONS

Experiment 1: Uniform percentage of gamma isomer on seed weight

Four batches, each of 25 seeds of Marquis wheat, were treated with the following materials: crude benzene hexachloride containing 10–12 per cent gamma isomer, a dust preparation known as Bennexane 50 containing 6 per cent gamma isomer, and "pure" gamma isomer. The rates of application of each material were such as to give 0.2, 0.4, 0.6, 0.8, and 1.0 per cent of the gamma isomer on the weight of the seed. These dosages were selected on the basis of the work of Thomas and Jameson (19), who obtained 50 per cent mortality of wireworms with applications of 0.28 lb. of gamma isomer to the acre. At normal prairie seeding rates, this would correspond to about 0.5 per cent gamma isomer on the weight of seed. Lower rates than these may well prevent damage or control wireworms (8), for which the rates recommended are from 0.025 to 0.2 per cent; but the adverse effects on plants are roughly proportional to concentration and can best be studied over this dosage range. To ensure retention of the higher dosages of the 12 and 6 per cent materials, a sticker was necessary; the material ected was Methocel (methyl cellulose) 100 c.p.s., which was applied as a er cent solution to all treated seeds. Check batches of untreated seed, d seed treated with Methocel only were also prepared.

Seed was treated at the end of March, and on April 8, 12 seeds from each of the seventeen batches were planted out in soil, six in each of two 6" pots, placed for germination in a humidified chamber at room temperature and given abundant water. The remaining seeds from the check batches, and from the highest and lowest dosage rates with each material, were placed for germination on wet filter paper in petri dishes in the dark at 24° C. Observations made on these tests follow in Tables 1 and 2, and Figure 1 shows the appearance of the seeds with the heaviest doses.

Two months after treatment, the remaining treated seeds, which had been stored in the dark, were also set for germination on filter paper. No noticeable differences resulted from the longer storage after treatment.

All batches except the checks showed the symptoms described by other workers in some degree, although these were least pronounced in the seeds treated with pure gamma isomer. Browning and swelling of the root tip, shortness of roots, absence of root hairs, and the short, fat, and flattened coleoptile, were exhibited in some degree throughout. The effects in the coleoptile appear, as Kostoff has stated (11), to be due to changes in the structure of the cells themselves rather than in their distribution and arrangement, epidermal cells appearing as large blisters.

On May 26, 48 days after planting, one series of pot plantings was examined for root growth, and all batches containing living plants from the second series were planted out in open ground. Root growth in the check pots was such that the entire soil content of the pot was closely tied together. In no other pot did the root development approach this, best growth being found from seeds treated with pure gamma isomer at the lowest dosage, in which the roots had just reached the bottom of the pot. Many of the seeds treated at the higher dosages showed no root development at all.

TABLE 1.—PETRI DISH GERMINATION OF WHEAT SEED TREATED WITH BENZENE HEXACHLORIDE PREPARATIONS, 12 SEEDS IN EACH BATCH. (EXPERIMENT 1.)

Treatment	Germination	Root hairs	Av. length coleoptile mm.	Av. length central root mm.
Crude BHC				
0.2% on seed weight	Complete	Nil	2.5	5.0
1.0%	2 failed	Nil	2.0	2.0
Bennexane 50 dust				
0.2% on seed weight	Complete	Nil	2.5	4.5
1.0%	5 failed	Nil	2.0	3.0
Pure gamma isomer				
0.2% on seed weight	Complete	Normal	4.5	15.0
1.0%	Complete	Normal	5.0	10.0
Checks				
Untreated	Complete	Normal	18.0	55.0
Methocel	1 failed	Normal	35.0	[illegible]5.0

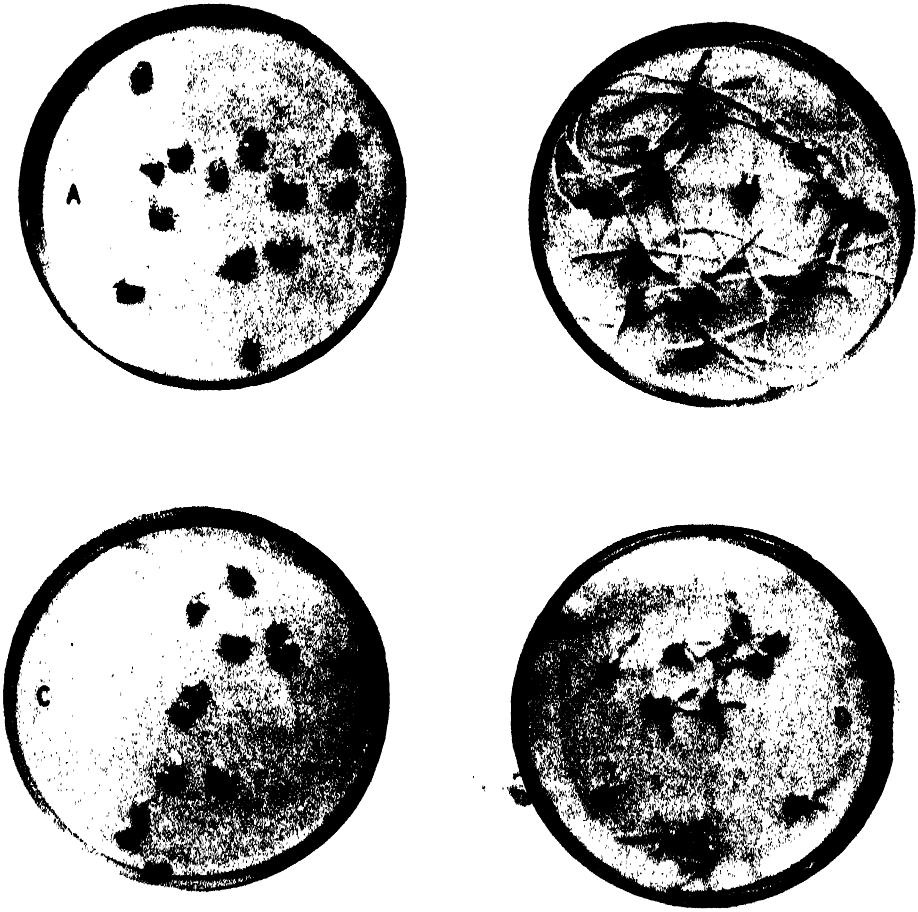

FIGURE 1. Wheat seed germinated after treatment with heavy doses of: A. Crude BHC; B. Check, untreated; C. A dust preparation containing 6 per cent gamma isomer; D. Pure gamma isomer.

TABLE 2.—POT PLANTING OF WHEAT TREATED WITH BENZENE HEXACHLORIDE PREPARATIONS, 12 SEEDS IN EACH TREATMENT, IN TWO BA[illegible]F SIX.
(EXPERIMENT 1)

Material	Per cent gamma on seed weight	Growth in pots			Transferred to open	
		5 days	12 days	48 days	June 21 (75 days)	Aug. 10 (125 days) No. of heads
Crude BHC	0.2	Nil	6 very small	2 normal (4 died)	2 plants heading out	3 very small
	0.4	3 distorted	6 very small	1 normal		
	0.6	1 distorted	2 very small	Nil		
	0.8	Nil	Nil	Nil		
	1.0	Nil	Nil	Nil		
Bennexane 50	0.2	Nil	4 very small	3 normal	3 plants no heads.	4 green
	0.4	Nil	7 very small	Nil		
	0.6	Nil	1 very small	Nil		
	0.8	Nil	3 very small	Nil		
	1.0	Nil	Nil	Nil		
"Pure" gamma isomer	0.2	8 normal	9 normal	9 normal	9 plants heading out	4 very small, 6 small green
	0.4	3 distorted	5 normal, 2 small	5 normal, 1 small	5 plants heading out	4 ripe, 2 green
	0.6	6 distorted	9 normal	7 normal	7 plants heading out	9 small, 3 green
	0.8	2 distorted	4 normal, 3 small	2 normal, 2 small	3 plants heading out	5 small, 7 very small green
	1.0	3 distorted	1 normal, 4 small	2 small	All plants died	
Checks **Methocel**	Nil	Normal germination and growth			10 plants heading out	9 ripe, 5 green
Untreated	Nil	Normal germination and growth			10 plants heading out	7 ripe, 3 green

Observations were made on the plants transferred to open ground on June 21, and on August 10 when the ears were collected. Seed collected was set for germination 6 months later; early development showed no abnormalities.

Petri dish germination tests were also made using the commercial preparation Benesan E2, a combined mercurial and BHC seed dressing containing 40 per cent gamma isomer, at 0.2 per cent gamma on seed weight, the maximum recommended rate, and at 0.4 per cent.

The lower dosage caused considerable distortion but no reduction in germination; at the higher dosage germination was also affected.

Experiment 2: Uniform percentages of total BHC on seed weight

Batches of 25 seeds of Marquis wheat were treated with the materials used in Experiment 1, to give with each material, the following percentages of total BHC on the seed weight: 0.2, 1.0, 1.8, and 9.0. These percentages are equivalent to the lowest and highest dosages of pure gamma (0.2 and 1.0 per cent) and of crude BHC (1.8 and 9.0 per cent) used in Experiment 1. Fifteen seeds from each batch, and fifteen check seeds, were planted out in the soil on May 26; the remaining seeds were placed for germination on wet filter paper in petri dishes at the same time. In the petri dish germination, seeds treated with pure gamma isomer were slightly in advance at the very early stages, but no other differences between the three materials were observed. Observations were made on plants in the open on June 4 and 21, and on August 10, and are presented in Table 3.

TABLE 3.—GROWTH OF WHEAT SEEDLINGS IN THE OPEN, FROM SEED TREATED WITH BENZENE HEXACHLORIDE PREPARATIONS, 15 SEEDS IN EACH BATCH. (EXPERIMENT 2)

Material	Dosage: % total BHC on seed	Growth on:		
		June 4 (9 days)	June 21 (26 days)	Aug. 10 (76 days) No. of heads
Crude BHC	0.2	Nil	Nil	4 plants, 1 small
	1.0	Nil	2 plants	3 plants
	1.8	Nil	Nil	Nil
	9.0	Nil	Nil	1 small plant
Bennexane 50	0.2	Nil	8 small plants	8 plants, 8 green
	1.0	Nil	3 small plants	1 small plant
	1.8	Nil	Nil	1 small plant
	9.0	Nil	Nil	Nil
Pure gamma isomer	0.2	Nil	2 small plants	2 plants, 2 green
	1.0	1 small plant	Nil	Nil
	1.8	1 small plant	1 small plant	Nil
	9.0	Nil	Nil	Nil
Check (untreated)	Nil	1 normal	7 normal	8 plants, 7 green

EXPERIMENTS WITH CONSTITUENTS AND BREAKDOWN PRODUCTS OF BHC

Experiment 3: Isomers of BHC and a mixture of trichlorobenzenes

This experiment was conducted on the same lines as Experiment 1 except that only petri dish germination tests were used. The materials tested were the alpha and beta isomers, two samples of gamma isomer, one freshly prepared, melting point 110–112° C., and a second deteriorated, melting point 90–100° C.; and a mixture of trichlorobenzenes prepared from the alpha BHC and consisting of at least 85 per cent of the 1,2,4-isomer. Several authors (6, 12, 16) have shown that BHC breaks down to give, among other substances, trichlorobenzene and hydrochloric acid.

Batches of fifty seeds each of Thatcher wheat were treated with methocel, followed by each of these substances at 0.2, 1.0, and 5.0 per cent on the weight of seed, the trichlorobenzene mixture after emulsification in distilled water with Triton X-100. Germination tests were repeated four times,

TABLE 4.—PETRI DISH GERMINATION OF WHEAT SEED TREATED WITH VARIOUS CONSTITUENTS OF CRUDE BENZENE HEXACHLORIDE. (EXPERIMENT 3.)

Treatment material	Per cent on seed weight	Germination in tests				Total	Remarks
		1	2	3	4		
Alpha BHC	0.2	8/10	10/10	8/10	8/10	34/40	Slight deformity especially at higher dosages in tests 1, 2, and 3. Pronounced deformity in 4.
	1.0	8	8	7	7	30	
	5.0	8	6	9	7	30	
Beta BHC	0.2	6	9	10	7	32	Normal development in all tests.
	1.0	5	10	10	8	33	
	5.0	8	10	10	6	34	
Pure gamma BHC	0.2	7	9	10	7	33	Typical deformation; more severe at heavier dosages, and particularly severe in test 4.
	1.0	8	9	9	6	32	
	5.0	7	8	7	5	27	
Deteriorated gamma BHC	0.2	7	8	9	6	30	Different from pure gamma in degree only; 5% and 1% approx. equivalent to pure gamma at 1% and 0.2% respectively.
	1.0	10	10	6	5	31	
	5.0	8	4	10	5	27	
Trichlorobenzenes	0.2	7	5	5	10	27	Germination somewhat slow, but when it occurred, normal development followed. At higher doses a white pulpy mass surrounded by brown discoloration developed at the normal point of emergence of the radicle.
	1.0	0	0	0	1	1	
	5.0	0	0	0	0	0	
Check	—	9/10	9/10	10/10	10/10	38/40	Normal development.

once immediately after treatment, again after 10 and 20 days' storage in the dark, and finally after a further 10 days' storage exposed to daylight under glass, during which time the seed was exposed to sunlight for about 12 hours. The first two tests were run at a temperature of 24° C., and the last two at day temperatures of 20° C. and night temperatures of 15° C. Ten seeds from each batch were used in each test. The results of these tests are recorded in Table 4. In addition to increased phytotoxic effects after insolation, a pronounced increase in the characteristic musty odour of the alpha and both samples of gamma isomer was noticed.

Experiment 4: Gamma BHC, trichlorobenzenes and HCe in the vapour phase

In Experiment 3 the following materials show distinct physiological activity: pure gamma BHC, deteriorated gamma BHC, and the mixture of trichlorobenzenes. A further experiment was done with these three materials and hydrochloric acid in which the germinating seeds were

TABLE 5.—PETRI DISH GERMINATION OF WHEAT SEED EXPOSED TO THE VAPOUR PHASE OF VARIOUS CONSTITUENTS OF CRUDE BENZENE HEXACHLORIDE. (EXPERIMENT 4.)

	Germination		Remarks
	1	2	
Pure gamma BHC vapour	10	8	Mean root length reduced 30% by comparison with check. This may be regarded as statistically significant, the probability of chance occurrence being less than 0.02. No other obvious symptoms of deformation.
Check	10	10	Normal development.
Deteriorated gamma BHC vapour	10	7	Mean root length reduced 58% by comparison with check, significantly greater than the reduction with pure gamma. Germination slow. No other obvious symptoms of deformation.
Check	10	10	Normal development.
Trichlorobenzenes vapour	0	0	No signs of any development at all.
Check	10	10	Extreme deformation of every seedling, typical of heavy seed treatment with solid gamma BHC.
Trichlorobenzenes vapour in sealed containers	0	0	No signs of any development at all.
Check	10	9	Normal development.
Trichlorobenzenes vapour 24 hr. dose	8	9	Typical gamma BHC seed treatment deformation in every embryo.
Check	10	10	Normal development
Hydrochloric acid vapour	10	9	Normal development.
Check	10	10	Normal development.

exposed only to the vapour phase. Ten seeds were used in each batch, and a separate check batch was used for each material. Watch glasses, each containing a liberal quantity of the appropriate material, were placed in the petri dishes in which the seeds were set for germination. Germination was carried out in the dark at a temperature of 24° C., each test batch accompanied by its check batch, and isolated from the other test batches.

Owing to the surprising results obtained in the check batch of seeds associated with the trichlorobenzene mixture (see Table 5), this experiment was repeated, and the most stringent precautions against contamination of the check batches were taken; the results were identical. Finally, two further repetitions of the trichlorobenzene test alone were made; first, using containers with airtight closures, and second, in petri dishes in which only a very small amount of trichlorobenzene was used, an amount which had completely volatilized within 24 hours.

Experiment 5: The vapour of trichlorobenzene isomers on several varieties of cereals

In this experiment the seeds were germinated on washed sand in large petri dishes. The treatment material was placed in a watchglass at the centre of the dish, and the seeds of the different varieties were separated from each other by radial strips of cardboard, the batches arranged in a random manner around the periphery. Six representative varieties of wheat, (Durum, Rescue, Thatcher, Marquis, Red Bobs and Kharkow) three varieties of oats, (Ajax, Victory and Larain) and three varieties of barley, (Montcalm, Newal and O.A.C. 21) were used. The materials used for the treatment were the 1, 2, 3, and the 1,3,5-isomers of trichlorobenzene, prepared by chlorination of paranitroaniline followed by diazotisation and further chlorination, and presumably free of other isomers, and the material described under Experiment 3, containing at least 85 per cent of the 1,2,4-isomer. A fourth batch with no treatment was run as a check. Germination was at 27° C.

No germination whatsoever occurred in any variety exposed to the vapour of the 1,2,4-isomer. In the vapour of the 1,2,3-isomer, all varieties showed some germination except for the wheats Durum and Kharkow, and the oats Ajax and Victory. After 8 days, however, all plants were dead. It is noteworthy that the only oat variety to germinate was Larain, which also made the best growth in the vapour of the 1,3,5-isomer. In the vapour of the 1,3,5-isomer, all varieties germinated and although growth was very much retarded in comparison with that in the check batches, the plants survived and all were living after 12 days.

Among the wheat exposed to the 1,3,5-isomer, Durum was retarded appreciably more than the other varieties in the early stages, but after 8 days there were no detectable differences. Among the oats, Ajax and Victory were appreciably more retarded than Larain; and among the barleys O.A.C. 21 was most retarded, and Montcalm least.

A sample of pure 1,2,4-isomer proved unobtainable, but confirmatory tests were run with crude material from two other sources: (1) prepared by the chlorination of benzene, and reported to "contain about 95 per cent 1,2,4-trichlorobenzene, most of the impurities consist of isomers of tetrachlorobenzene, with very low percentages of dichlorobenzene isomers", and

(2) "1, 2, 4-trichlorobenzene and 5 to 10 per cent of the 1,2,3-isomer—boiling range in the neighbourhood of 2 to 2½° C. from initial boiling point to dry point; orthodichlorobenzene and paradichlorobenzene are probably not present, and we are fairly certain that the tetrachlors are not present either,—the material contains no free hydrochloric acid." No differences could be detected in the reactions to the original sample and to these two further samples.

In view of the fact that 2,4-dichlorophenol, a component of the 2,4–D molecule, has been mentioned as a breakdown product of benzene hexachloride (14), seeds were also germinated in various concentrations of the vapour of this material. The reactions were closely comparable to those obtained with 1,2,4-trichlorobenzene.

DISCUSSION

The results of Experiment 1 are anomalous; they suggest that the deformation of the seedlings is neither due to the gamma isomer as such, nor yet definitely due to any other major constituent. Analysis and melting point determinations made on the gamma isomer material after these tests indicated that this was very close to 100 per cent gamma isomer. Possible explanations of the greater effect obtained with the preparations of crude benzene hexachloride are: first, that the effect is a non-specific one, due to benzene hexachloride in any form; this is unlikely, in view of the very different physical characteristics and probably spatial arrangement of the molecules, and the similar severity of symptoms obtained with the 50 and 100 per cent preparations. A second possibility is that the effect is due to an impurity operating at very low concentration, perhaps a volatile breakdown product either of several of the isomers, or of the gamma isomer alone. Such a volatile product would be maintained at higher concentration in the proximity of the seed by the heavier coating of other isomers and diluent on seeds treated with preparations of the crude material, which would account for the more severe symptoms with these preparations.

The practical conclusions from Experiment 1 are, first, that if seed treatment with BHC is practicable at all, a refined product with high gamma content is desirable; second, that the margin of safety between a dose which will control wireworms, and a dose which will imperil the plant, at least under difficult moisture conditions, is probably none too great. Although germination and growth of the next generation is not affected, as Kostoff (11) has shown, the integrity of a strain would be threatened by repeated treatments.

Experiment 2 was designed to decide finally if the effect was non-specific and due to BHC in whatever form. The soil germination tests suggest that this is not so, seed treated with pure gamma isomer giving a markedly poorer stand than that treated with preparations of the crude material. The earlier appearance of seedlings from seed treated with pure gamma isomer is peculiar; in the petri dish tests a similar effect was noticed, but no other conclusions could be drawn from these tests. It should be mentioned that while this experiment might be expected to distinguish between a non-specific activity of BHC in any form, and activity of the gamma isomer, if the activity were due to a breakdown product, the ages of the respective samples might be the more important factor.

The results of Experiment 3 also appear somewhat anomalous. The beta isomer may be regarded as physiologically inactive and the alpha isomer almost so; this finally establishes the fact that the effect is not a non-specific activity of BHC. The two samples of gamma isomer each produced what may be regarded as typical "gamma deformation" symptoms, while the mixture of trichlorobenzenes had a pronounced effect on germination, inhibiting this completely at the two higher dosages, and reducing it to about 50 per cent at the lowest dosage. The normal development of the seeds germinating at the lowest dosage of trichlorobenzenes suggests that these are two separate and distinct effects. The distinctly more severe deformation symptoms given by the deteriorated sample, containing a lower proportion of gamma isomer, again suggest however that these symptoms are caused, not by the gamma isomer itself, but by a breakdown product of this. Breakdown, to give this product, appears to be accelerated by exposure to sunlight, which also favours the production of materials responsible for the characteristic odour of crude BHC.

Experiment 4 was designed to test the suggestion advanced in the discussion on Experiment 1, that a volatile breakdown product was the physiologically active material. As so often happens, a fortuitous occurrence in this experiment provided a clue to the solution of the problem as a whole. The consistent deformation obtained in the untreated checks accompanying trichlorobenzene treatments, viewed in the light of the results in sealed containers and with limited dosage, was clearly due to the vapour of the active material diffusing out from under the cover of the treatment petri dish and into the adjacent check petri dish, in sufficient concentration to produce typical "gamma deformation" without affecting germination. This also indicates that the two apparently separate effects described in the previous paragraph are really identical, inhibition of germination being the ultimate form of the symptoms. The results obtained with the two gamma isomer preparations confirm those in Experiment 3.

This demonstration of volatility of the phytotoxic principle also permits an explanation of the absence of deformation at the lowest dosage of trichlorobenzene in Experiment 3; the active material had presumably been dissipated by volatilization before growth had commenced, and in the absence of gamma isomer no more was produced.

Gavaudan and Gavaudan (4) have demonstrated similar deformation of wheat seedlings germinated in air saturated with the vapour of a number of different materials, among them 1,3,5-trichlorobenzene. Experiment 5 was designed to show whether this, or some other material, was mainly responsible for the phytotoxic effects observed with BHC, and at the same time to see whether varietal differences in response to these materials existed.

1,2,4-trichlorobenzene appears to be by far the most important phytotoxic material, although considerable toxic effect is produced by the 1,2,3-isomer and some by the 1,3,5-isomer. It appears likely that the differences are an expression of differences in vapour pressure, and that the molecular toxicities of the three materials are similar. The formation of 2,4-dichlorophenol may be of some significance, and is particularly interesting inasmuch as Meunier (14) describes the odour of the mixture of chlorophenols which he obtained in terms which would apply remarkably well to that of crude

BHC. In normal conditions, a mixture of these phytotoxic materials appears to be produced in most effective proportions and amount from the least stable gamma isomer, in threshold amounts from the alpha isomer, and in quite unimportant amount, if at all, from other isomers. The varietal differences do not appear sufficient to justify plant breeding recommendations, although Durum wheat is possibly peculiarly susceptible, and Larain oats and possibly Montcalm barley are somewhat resistant.

As other workers have observed (9), damage to seedlings tends to be much less in soil germination than in petri dishes. This may be partly attributable to adsorption of toxic materials by soil particles, and partly to better ventilation. It should be noted that poor moisture conditions, when impaired root development is of the greatest importance, are normally associated with good soil ventilation. The work of Smith (17) would seem to suggest that slower breakdown under soil conditions may also be a factor.

The very small amounts in which the 1,2,4-trichlorobenzene is effective, suggest that it might have applications in the field of weed control, possibly if incorporated in place of 2,4-dichlorophenol in a molecule of the 2,4-D type.

Although the literature does not adequately reflect this, BHC has often given anomalous results as an insecticide; some of these results may be explicable on the basis of differences in breakdown reactions under different conditions. Trichlorobenzenes have been shown to have some insecticidal properties themselves (7, 15) and Guilhon (5) has shown crude BHC to be more toxic than its content of gamma isomer would suggest.

SUMMARY

Wheat seed was treated with various preparations and constituents of crude benzene hexachloride, and germinated to decide which of these constituents was responsible for the deformation of the seedlings.

This deformation is not due to the accepted insecticidally active principle, the gamma isomer. A mixture of trichlorobenzenes prepared by the breakdown of alpha BHC possesses this activity in far higher degree, relatively small doses causing the ultimate symptom—inhibition of germination. The material is active in the vapour phase. The most active material in this mixture is 1,2,4-trichlorobenzene, followed by the 1,2,3, and lastly the 1,3,5-isomer. 2,4-dichlorophenol, which may also be produced in the breakdown of BHC, causes similar deformation. The possibility of utilizing these materials in the role of selective weed killers is under investigation.

BHC preparations for cereal seed treatment against wireworms should be materials of high gamma content, applied at the minimum practicable rate for control. The hazards accompanying their use are likely to be increased by storage after treatment, especially if ventilation is inadequate, by exposure to sunlight, and by deficient moisture and high pH of the soil. Damage seems particularly likely to occur with Durum wheat; among oat varieties tested Larain suffered least damage, and among the barleys, Montcalm appears to have some resistance.

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EFFECTS OF ESTROGEN AND ANDROGEN ON LIVER IRON OF THE IMMATURE PULLET[1]

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INTRODUCTION

Sex is known to influence the storage and utilization of iron. Mitchell (9) showed that female rats had a higher blood hemoglobin than male litter mates and that females also took longer to become anæmic. Wakeham and Halenz (16) found that the livers of female rats contained a greater concentration of iron than the livers of male rats of the same age. Smith and Otis (14) found that hemoglobin regeneration was more rapid in female rats than in male rats. Otis and Smith (10) subsequently showed that the total iron per gm. body weight was greater in female rats than in males in spite of the smaller size of the female rats. Rose and Hubbell (12) found that female rats stored some 12 per cent more iron than males when the intake per gm. body weight was the same for each sex. The foregoing observations are all consistent with the idea that female rats have a greater capacity for storing iron than do males.

It was natural to suspect that gonadal hormones might be involved in determining such a tendency. Some years ago Steenbock *et al.* (15) reported that injections of estradiol benzoate (Progynon-B, Schering) into sexually immature female rats resulted in an increased storage of iron as compared with the storage by unestrogenized female litter mates. Steenbock *et al.* (15) did not furnish quantitative date in their report, but Widdowson and McCance (17) have recently published data relating to sexual differences in the storage and metabolism of iron in a number of vertebrate species. Widdowson and McCance (17) found that the livers of cockerels contained 8.7 mgm. inorganic iron per 100 gm. at twenty weeks of age, whereas the livers of comparable pullets contained 14.7 mgm. inorganic iron per 100 gm. liver. The variability of the data was large, but the difference between the average values for the sexes was statistically significant. The foregoing findings suggested that it would be of interest to explore further the possible effects of estrogen and androgen upon the iron content of the livers of the sexually immature pullet. The present paper deals with experiments made with this end in view. The possible effects on the copper content of the livers were examined at the same time.

EXPERIMENTAL

Method of Experiment

The experimental birds were sex-linked cross-bred (New Hampshire ♂ x Barred Plymouth Rock ♀) pullets. Two similar experiments were carried out. In each experiment sixteen birds were used. Each sixteen birds were distributed at random into four groups of four birds each and housed at random in individual cage laying batteries. Group A served as controls; Group B received androgen only; Group C received estrogen only; and Group D received both androgen and estrogen. The estrogen was estradiol benzoate (Progynon B, Schering) and the androgen was testosterone propionate (Oreton, Schering). Each treatment was divided into six equal doses which were injected into the breast muscles on alternate days over a twelve-day experimental period. The total amounts of oily base injected were equalized as between all the pullets. The levels of treatment are given in Table 1. The androgen treatment given to Group D was designed to produce a degree and speed of hypertrophy of oviduct and comb of the same order as that which takes place as the pullet enters normal reproductive activity. All birds in each experiment were given the same amounts of the all-mash diet (a commercial growing ration) so as to avoid effects due to differences in food intake. The birds were decapitated and bled on the second morning after the last injection, and the livers removed for analysis.

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Analytical Methods

Total iron was determined by a slight modification of the A.O.A.C. method (1). About 1 gm. liver was ashed with sulphuric and perchloric acids and after suitable dilution an aliquot was taken for colour development using $\alpha\,\alpha'$-dipyridyl.

The inorganic iron of tissues corresponds to iron extractable from the fresh tissue by mildly acid solutions. It has been variously described as ionizable iron (13), available iron (4), inorganic iron (17) and free iron (6), and different workers have described different procedures for estimating this fraction of the tissue iron. For the purposes of the present experiments inorganic iron was determined by the method of Shackelton and McCance (13) modified for photoelectric colorimetry as follows:

About 0.75 gm. liver was weighed into a small mortar and crushed with a pestle. The liver was washed into a 25 ml. glass stoppered cylinder, using four 5 ml. portions of Walpole's acetic acid-sodium acetate buffer at pH 5.5 (5). The cylinder was then heated in a boiling water bath for ten minutes. After cooling, 0.5 gm. sodium hydrosulphite was added. The contents of the cylinder were mixed, and 2 ml. of 0.1 per cent $\alpha\,\alpha'$-dipyridyl was added. The cylinder was well shaken and left to stand overnight. Five ml. ethanol was added, the cylinder was well shaken and again left to stand overnight. The solution was filtered into a 100 ml. volumetric flask, and the cylinder washed out with distilled water. The residue on the filter paper was repeatedly washed with distilled water until a volume of 100 ml. was obtained. This solution was solution D.

At the same time three additional solutions were prepared. Solution A contained 20 ml. buffer, 0.5 ml. sodium hydrosulphite and 5 ml. ethanol. It was filtered and made to 100 ml. by washing the filter paper. Solution B contained the same amounts of buffer, hydrosulphite and ethanol plus 2 ml. 0.1 per cent $\alpha\,\alpha'$-dipyridyl and was similarly filtered and made up to 100 ml. Solution C was prepared in the same way as solution A, using 0.75 gm. liver, but omitting the addition of $\alpha\,\alpha'$-dipyridyl.

The Evelyn colorimeter was set at 100 per cent transmission (440 m u filter) with solution A, and solution B was read. This gave the photometric density due to reacting iron in the reagents and filter paper. The colori-

TABLE 1.—EFFECTS OF ESTROGEN AND ANDROGEN ON THE IRON CONTENT OF THE LIVER OF THE SEXUALLY IMMATURE PULLET

Average results for Experiments 1 and 2

Number of pullets	Group A	Group B	Group C	Group D
	8	8	8	8
Total dosage of extradiol benzoate, mgm.	Nil	Nil	18	18
Total dosage of testosterone propionate, mgm.	Nil	6	Nil	6
Initial weight, kgm.	1.01	1.03	1.01	1.08
Final weight, kgm.	1.22	1.24	1.22	1.23
Food consumption, kgm.	0.92	0.91	0.92	0.92
Liver weight, gm.	20.4	21.4	32.4	32.1
Liver inorganic iron:				
mgm. per 100 gm. liver	14.8	10.1	12.6	11.4
mgm. per liver	3.0	2.2	4.1	3.7
mgm. per kg. live weight	2.5	1.8	3.4	3.0
Liver total iron:				
mgm. per 100 gm. liver	18.4	12.8	16.0	14.9
mgm. per liver	3.8	2.7	5.2	4.8
mgm. per kg. live weight	3.1	2.2	4.3	3.9
Liver inorganic iron as per cent of total iron	81	79	79	77

	Least significant difference between groups ($P = 0.05$)	c.v. per cent
Liver inorganic iron:		
mgm. per liver	1.2	32.7
mgm. per kg. live weight	0.8	27.9
Liver total iron:		
mgm. per liver	1.5	31.4
mgm. per kg. live weight	1.0	29.1

meter was then set at 100 per cent transmittance with solution C, and the unknown solution D was read. This gave the photometric density due to reacting iron in the unknown plus reacting iron in the reagents and filter paper. The latter photometric density was corrected by deducting the photometric density due to iron in the reagent and filter paper.

The concentration of iron corresponding to this photometric density was then read from the calibration curve prepared by plotting iron concentration against photometric density of the $\alpha\alpha'$-dipyridyl complex. The reaction obeys Beer's Law for concentrations ranging up to 1.8 micrograms iron per ml. of final reaction mixture.

Copper was determined by digesting 1.5 gm. liver with 2 ml. concentrated sulphuric acid and successive additions each of 1 ml. perchloric acid until a clear digest was obtained. The solution was diluted and transferred quantitatively to a 100 ml. volumetric flask. The copper content of a suitable aliquot was determined by the method of Boulet and McFarlane (2).

Experimental Results

In the first experiment the pullets were 92 days old when killed and in the second experiment they were 103 days old when killed. Treatments and experimental procedure, however, were the same in both experiments Accordingly the data for the entire 32 pullets have been considered together and are presented in Table 1. The analysis of variance was made by the "split plot" method.

Variability of Liver Iron

The results reveal a fairly wide variability in both total iron per liver (c.v. = 31.4 per cent) and in inorganic iron per liver (c.v. = 29.1 per cent). This variability extended to both controls and treated birds. The data presented by Widdowson and McCance (17) also display a wide variability in the liver inorganic iron of both the pullets and the cockerels which they examined. The percentage of the total iron present as inorganic iron did not display such a wide degree of variability. It would appear that the iron content of the fowl's liver is subject to wide variation even in the case of pullets of the same strain and hatching and which have been reared under the same conditions.

Effect of Androgen on Liver Iron

A comparison of the data for Group A (control) and for Group B (androgen only) suggests that androgen tended to give smaller concentrations of both total iron and inorganic iron in the liver. Androgen also appeared to reduce both the total iron per liver and the inorganic iron per liver, and also the liver total iron per kgm. live weight. While none of these differences attained significance at the 5 per cent point, they approached such a level sufficiently closely to be regarded as on the borderline of significance. Androgen did not appreciably affect liver weight.

A comparison of Groups C (estrogen only) and D (androgen + estrogen) suggests that androgen evoked similar trends in liver iron in the presence of estrogen as well as in its absence; but here again the differences did not reach significance at the 5 per cent point.

Effects of Estrogen on Liver Iron

Estrogen increased liver weight by approximately 50 per cent. Estrogen also tended to decrease the concentration of both inorganic and total iron in the liver, but not to a significant degree.

None of the treatments gave significant differences in live weights or live weight increases and the live weights of all groups were sensibly the same. When the amounts of inorganic and total iron per liver are considered, it will be noted that estrogen (Group C) increased the mg. inorganic iron and the mg. total iron per liver, but that one difference from the controls (Group A) failed to attain significance. However, when the data were calculated on the basis of mg. liver inorganic and total iron per kg. live weight, then estrogen gave a significant increase over the controls.

Estrogen plus androgen (Group D) gave significant increases of liver inorganic iron and total iron as compared with androgen (Group B) whether calculated as mgm. per liver or as mgm. per kgm. live weight. Moreover, estrogen also gave significant increases of liver inorganic iron and total iron as compared with androgen when calculated per liver as well as when calculated per kgm. live weight. It is clear from these results that estrogen increased the amounts of both inorganic and total iron in the liver.

Estrogen plus androgen (Group D) gave increases as compared with the controls (Group A), but these increases were smaller than the increases secured by estrogen alone (Group C) and did not attain significance.

The most reasonable explanation of the results as a whole is that the effect of androgen in bringing about a decrease of liver iron per liver and per kgm. live weight was real though not significant, and that this effect was exerted in the presence of estrogen as well as in its absence. Thus, when both hormones were administered together, the amounts of inorganic iron and total iron in the liver were intermediate between the amounts in the androgenized and in the estrogenized birds. Furthermore, although the treatment with estrogen plus androgen gave significantly greater amounts of total and inorganic iron in the liver than did androgen, it did not give a significant increase over the values for the controls. This fact, taken with the consistent trends of the average results, lends strong support to the foregoing explanation of the results as a whole.

The tendency for the concentration of iron in the liver to fall as a result of estrogen treatment is of interest. This feature of the results suggests that, in short-term experiments, hypertrophy of the liver may be relatively faster than the increase of storage of iron. It would be of interest to carry out similar experiments using more extended periods of treatment and lower levels of estrogen.

Effects of Estrogen and Androgen on Liver Copper

The effects of the hormonal treatments on liver copper were studied in the case of sixteen birds only (i.e., the livers from the second experiment). The results (Table 2), however, were sufficient to indicate that gonadal hormones do not affect liver copper to a degree comparable with the effects on liver iron.

TABLE 2.—EFFECTS OF ANDROGEN AND ESTROGEN ON THE COPPER CONTENT OF THE LIVER OF THE SEXUALLY IMMATURE PULLET

Number of birds	4	4	4	4
Total dosage estradiol benzoate mgm.	Nil	Nil	18	18
Total dosage of testosterone propionate mgm.	Nil	6	Nil	6
Initial weight, kgm.	1.08	1.07	1.11	1.11
Final weight, kgm.	1.25	1.26	1.28	1.26
Total food consumption, kgm.	0.90	0.91	0.91	0.92
Liver weight, gm.	21.3	22.0	33.6	33.7
Liver copper:				
mgm. per 100 gm.	0.58	0.47	0.34	0.34
mgm. per liver	0.11	0.10	0.11	0.10
mgm. per kgm. live weight	0.10	0.08	0.07	0.08

The results suggest a decrease in concentration of copper in the liver as a result of estrogen treatment; but when the data are expressed in terms of liver copper per kg. live weight, then there does not appear to be any difference between the copper contents of the livers of the control pullets and of the pullets receiving androgen, estrogen or androgen plus estrogen.

It may be observed that the copper content of an egg is of the order of one-tenth of the iron content. The results do not provide any evidence for the existence of copper reserves analogous with the inorganic iron of the liver.

DISCUSSION

In spite of the fact that some of the differences concerned do not attain statistical significance, the results as a whole afford strong support for the view that androgen tended to depress storage of iron in the liver, that estrogen increased storage of iron by the liver and that estrogen and androgen exerted their effects on the storage of iron by the liver independently. The results support the contention that the difference between the iron content of the livers of cockerels and pullets, as noted by Widdowson and McCance (15), is directly due to opposing effects of predominant androgen in the male and predominant estrogen in the female.

An average hen's egg contains about 2.3 mgm. iron concentrated mainly in the yolk (9) and there is little evidence that the diet of the hen has any effect on the iron content of the egg (7). Extensive data for the iron content of the fowl's liver do not appear to be available, and the principal studies relate to the changes in iron content during incubation (8). However, if it be assumed that the livers of the pullets examined by Widdowson and McCance (17) weighed about 50 gm. then these livers contained from 3.5 mgm. to 19 mgm. inorganic iron per liver. The inorganic iron is unlikely to represent less than about 75 per cent of the total iron (*vide* results of present experiments). The total iron content of their pullets' livers was, therefore, probably of the order of 4.7 mgm. to 25 mgm. iron per liver. It is thus evident that the iron requirements for egg production are fairly large in relation to the liver stores of iron. At the same time, and from a practical standpoint, McFarlane *et al.* (7) have pointed out that the possibility of nutritional anæmia in poultry, as a result of a deficiency of iron or copper in the diet, would seem to be extremely remote.

From the standpoint of iron metabolism in the pullet, it seems plausible to suggest that increasing estrogen activity will lead to an increased storage of iron in the liver as laying approaches. Any concurrent androgen activity will tend slightly to offset this influence, in contrast to the effects of androgen in relation to pre-laying storage of calcium in the pullet's skeleton (3). Furthermore, the amount of iron in the liver may well be subject to considerable fluctuations in relation to the demands for laying.

SUMMARY

1. The livers of immature pullets treated with estrogen contained significantly more total iron and inorganic iron per kgm. live weight than the livers of comparable pullets treated with androgen.

2. The livers of pullets treated with estrogen plus androgen contained significantly more total iron and inorganic iron than the livers of comparable pullets treated with androgen only.

3. The livers of pullets treated with estrogen only contained significantly more total and inorganic iron per kgm. live weight than the livers of comparable untreated pullets. Neither androgen alone nor estrogen plus androgen gave liver iron contents significantly different from those of the untreated pullets.

4. It is suggested that the results are most simply explicable on the ground that estrogen increases while androgen decreases storage of iron in the liver of the fowl, and that each hormone will continue to exert its influence on the storage of iron in the presence of the opposing influence of the other.

5. The copper contents of the livers of pullets treated with androgen or estrogen or both hormones did not differ appreciably from that of untreated pullets.

ACKNOWLEDGMENTS

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A FOUR-ROW POWER PLOT SEEDER

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In the dry area of west central Saskatchewan, served by the Dominion Experimental Station, Scott, the seed bed, in many springs, has a loose and dry surface with an underlying layer of hard clay, difficult to penetrate without power equipment. This condition in the spring had made it difficult to seed small grains deeply enough into moisture to ensure germination when using small, hand-operated nursery seeders. It is chiefly because of this soil condition in the spring that a decision was made to design and construct a power seeder in an endeavour to solve this soil-penetration problem. Other reasons influencing this decision were the difficulty of obtaining suitable extra labour for the short seeding period and the necessity of utilizing to the full the short period in the spring favourable for obtaining uniform stands of small grains in nursery plots.

In constructing the seeder, the following objectives were kept in mind:

1. To obtain sufficient penetration to place the seed in moist soil in the following types of seed beds: (*a*) those dry and powdery on top; (*b*) those difficult to penetrate because of clayey composition; (*c*) poorly prepared, trashy seed beds, and (*d*) seed beds on relatively new land containing clods and roots.

2. The seeding of four rows simultaneously to reduce labour costs and to speed up seeding operations.

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[1] Cerealist, Substation Supervisor and Plotman, respectively.

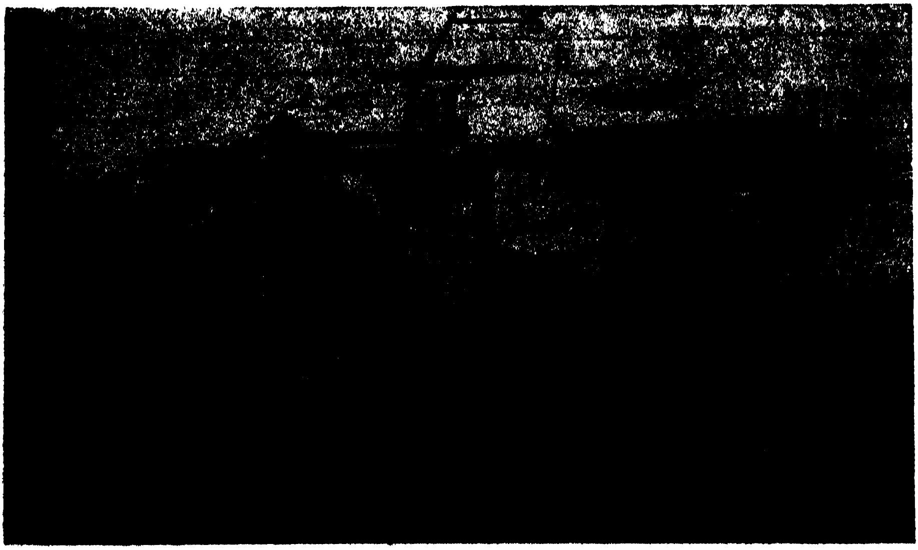

Figure 1. A side view with pertinent parts labelled. 1. Supporting arms. 2. Drawbolt. 3. Drawbar. 4. Arms for raising drawbar. 5. Pressure control lever. 6. Pressure arms. 7. Fulcrum of pressure arms. 8. Pressure bar. 9. Pressure springs. 10. Frame supporting V-belts. 11. V-belt unit. 12. 3/8 inch roller chain driving belts.

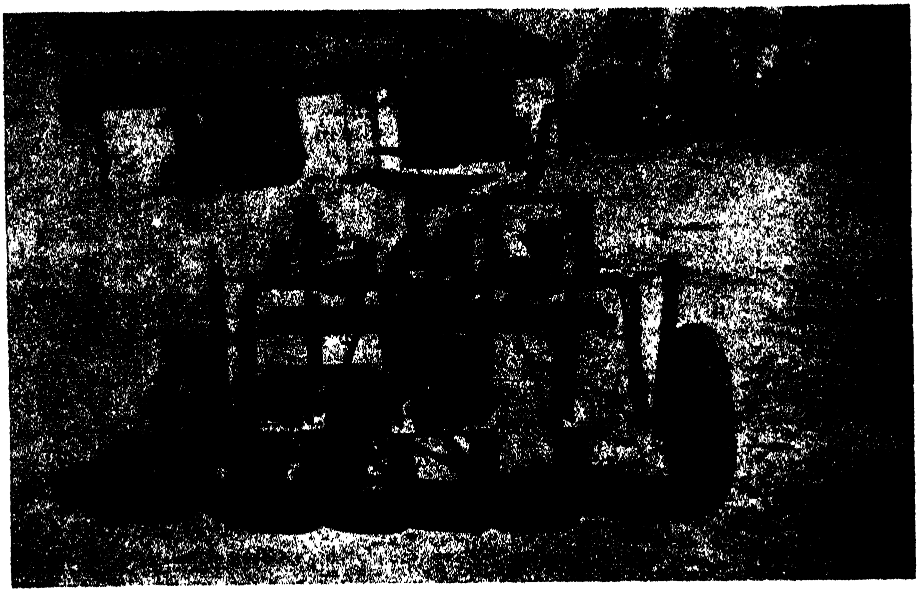

FIGURE 2. Rear view.

3. The use of four individual belts so that any desired type of plot i.e. single, two, three or four row plots, could be seeded.

4. Adjustable spacings for the furrow openers to seed nine-inch or foot spacings.

DESCRIPTION OF THE MACHINE

The assembled machine is shown in Figures 1 and 2 and different views of it in operation are given in Figures 5, 6, 7 and 8.

The 5 h.p. tractor was purchased. The double disk furrow openers with grain spouts, tension springs and supporting arms are the same as those used on a standard make of large grain drill. The V-belt attachment for delivery of the seed to the grain spouts consists of four Kemp (1) V-belt seeders, with wheels and handles removed, mounted on a 1½ inch angle iron frame, 22 inches × 38 inches, above the disks. The distinguishing feature of the Kemp V-belt seeder is an endless rubber belt, shaped in the form of a "V". This belt is stretched over two steel rollers, spaced 20 inches apart, thus giving 20 inches of belt over which seed is distributed by hand. The portion of the belt covered by the seed depends on the length of row to be seeded. The ratio of the belt speed to ground travel is 1 foot to 12 feet. Thus, in seeding 18½ feet of row, as is done in seeding a rod row, the seed is distributed over approximately 18 inches of belt. As the machine is moved over the ground the seed is carried by the belt to the conductor tube and thence to the furrow opener which places it in the soil. The V-belts which deliver the seed to the spouts are driven by a roller chain linkage from the hub assembly of the rear wheels to the axles of the V-belts. A chain on each side drives two V-belts. The lever and drawbar of the tractor are used to lower and lift the double disk furrow openers. This action is based on the fulcrum principle. As the drawbar is lifted, pressure is placed

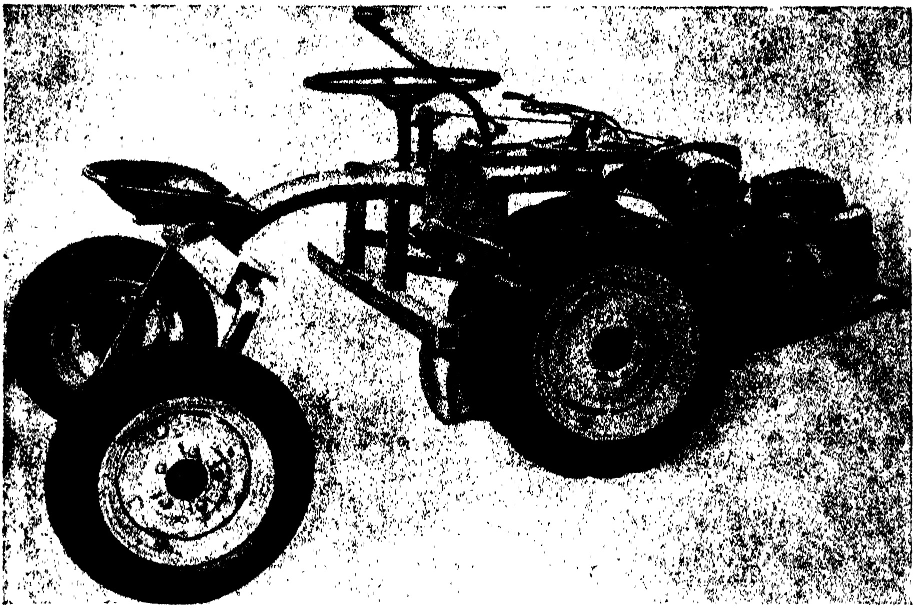

FIGURE 3. 5 h.p. riding garden tractor showing existing parallel linkage to tool-bar. The original tool-bar with two upright connections was replaced by the drawbar shown in Figure 4. The seeding attachment could be readily adapted to other styles of small 4-wheel tractors.

on the pressure springs to force the disks into the soil; as the drawbar is lowered, the disks are lifted clear of the ground. Figure 3 shows the tractor with original tool-bar.

An angle iron drawbar was made to take the place of the channel iron tool-bar which came with the tractor. Gusset plates were welded on to both ends and the middle of this drawbar and holes drilled in them through which a three-quarter inch rod was passed. This rod passed through the supporting arms of the double disk assembly to pull the disks. Three-quarter inch piping of appropriate lengths was used between the four supporting arms to provide different row spacings. On this machine, two spacings were used, viz., nine inch and one foot. Figure 4 shows this hook-up in detail.

The tractor is equipped with a multiple V-belt clutch which permits of instantaneous stopping and starting. After one season's work, seeding and cultivating, involving thousands of stops and starts, the clutch is still in first-class shape. When the clutch does wear out, replacement can be made easily and cheaply by the purchase of new V-belts. The machine is quite manoeuverable. It reverses and can be turned in its own length, which is approximately six feet. Speed is controlled by the throttle.

SEEDING PROCEDURE

Seed for each row is placed in a single envelope and these are laid out on a table in the laboratory in exactly the same way as they will be seeded in the field. As the seeding is done straight across all the ranges of the test, the envelopes are taken from the table for this manner of seeding in groups of four, stapled together and numbered in chronological order. The envelopes are then checked back with the field plan to see that everything is in order. When ready to seed, the groups of four envelopes are taken to the field and placed in the rack at the back of the drill in chronological order.

Six-foot pathways between ranges are marked out by driving a half-ton truck crossways every 18½ feet. In cultivated ground, the tires make a wide, easily discernible mark. In this way, the marking-off of ranges is done quickly and efficiently.

One man drives the tractor, using the mark of one of the rear wheels as a guide. Two men feed the V-belts with the packaged seed, each feeding two belts. One man can feed all four belts; but it was found that relatively greater efficiency could be obtained by using two men for feeding. The machine is stopped in each pathway without lifting the disks while the seed is spread on the belts from the envelopes. The length of belt required for the length of row to be seeded had been calibrated previously and an inclined stop placed over the V-belt to mark the length of belt over which the seed is to be spread and to prevent any vibration on the belt. The drill is stopped in the pathway far enough back from the range mark so that when the machine is set in motion the first seeds drop at this mark. A little practice by the driver of the machine will enable him to stop at the proper spot in the pathway.

OBJECTIVES OBTAINED

This four row power nursery seeder has achieved the objectives in mind when it was constructed. Excellent stands were obtained when seeding was done in heavy clay loam soils, in soils which were loose and dry at the surface, in seed beds which contained a considerable amount of trash and in poorly prepared seed beds in co-operative tests with farmers. Figures 9 and 10 show stands at two different stages of growth.

FIGURE 4. Details of hook-up of disk assembly to tractor drawbar (detached from tractor).

FIGURE 5. Two men feeding two V-belts each, and one driving tractor.

FIGURE 6. The drill half-way across a block of six rod row ranges.

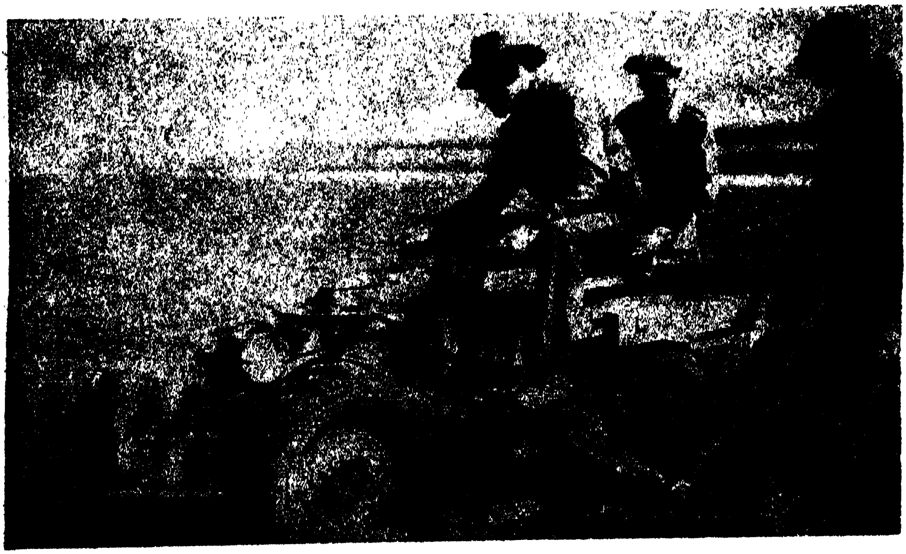

FIGURE 7. A side view of machine in operation, showing depth of penetration of double disk furrow openers.

FIGURE 8. Rear view of machine in actual seeding operation.

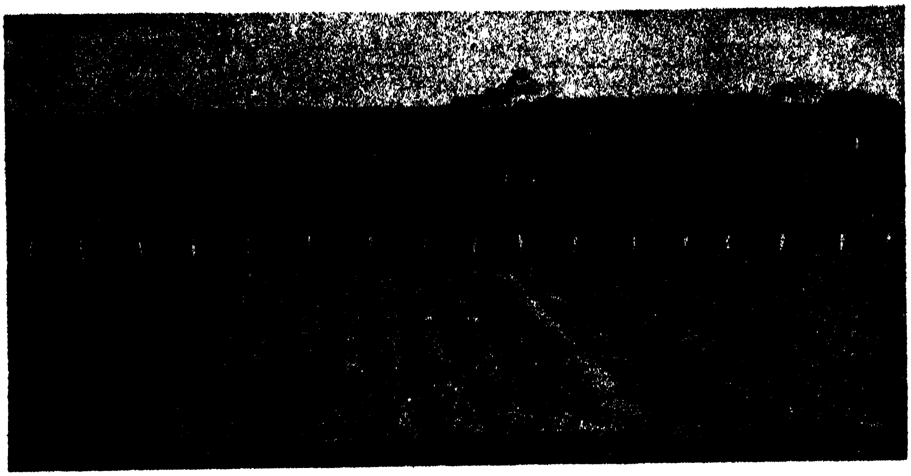

FIGURE 9. Stands at Experimental Station, Scott, Sask., three weeks after seeding.

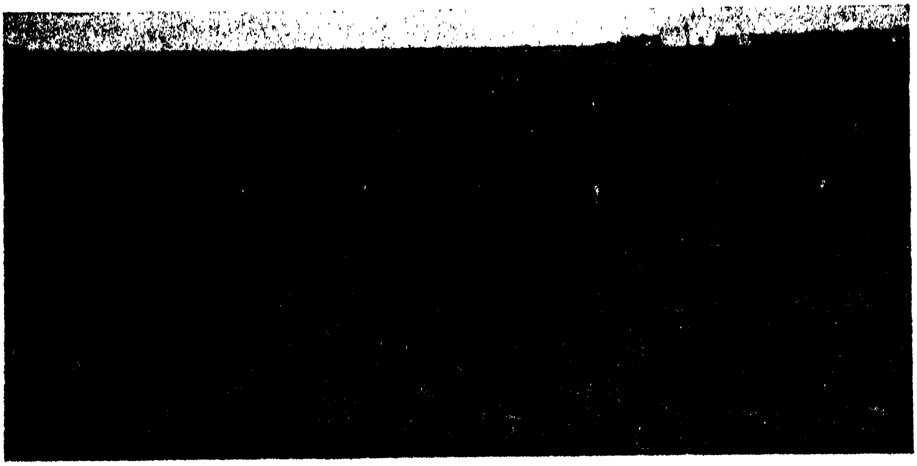

FIGURE 10. Stands at Experimental Station, Scott, Sask., five weeks after seeding.

Seeding was speeded up considerably and less labour was required. Previously, using three single-row hand-operated seeders, with two men to each seeder, six men seeded approximately 500 rows per hour. Using the power seeder and three men, from 500 to 600 rows were seeded per hour. Another desirable feature was the straightness of the rows with uniform spacings between rows, not usually obtained when single hand seeders were used. This proved a great advantage in weeding and harvesting.

SEEDING OUTSIDE TESTS

The seeder fitted quite nicely into a half-ton truck and was taken around the territory to seed outside tests. Its use resulted in a considerable saving of time away from the Station. A more important consideration, however, was the ability of this seeder to seed into rough, trashy and difficult-to-penetrate seed beds often encountered on farms where the seed bed had been prepared well enough for the use of large machinery but not well enough for the use of small nursery seeders.

CULTIVATION

The drill can be quite easily and quickly detached from the tractor when seeding operations are completed and cultivator shovels or weeding blades attached for weeding purposes during the summer. A three-foot weeder blade has proven very satisfactory in cutting off and weeding six-foot pathways. Weeds were controlled satisfactorily without too much loosening of the soil and the pathways were left firm under foot, resulting in firmer and less dusty footing when taking notes.

SUMMARY

1. A four row power plot seeder has been described. Such a seeder was conceived and constructed mainly to avoid the discarding of many cereal tests due to poor stands in years when small, hand-operated seeders were not capable of placing the seed deeply and uniformly enough into moist soil.
2. A small 5 h.p. garden tractor was used as the source of power and a small drill, capable of seeding four rows simultaneously, was built to be attached to this tractor.
3. It was found possible, with this seeder, to seed into any type of seed bed and to obtain uniform stands.
4. The seeder was small and compact enough to be loaded into a small truck, transported around the country and used for seeding tests away from the station.

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EFFECTS OF RADIATION ON THE TEMPERATURES OF INSECTAN HABITATS[1]

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INTRODUCTION

During 1947, a program of meteorological observations was organized to investigate environmental situations of interest to those concerned with field problems of forest entomology. Data accumulated pointed up certain aspects of micrometeorology which, though not unknown, have not been assigned special importance in the past. This paper deals with one of these aspects—the effects of solar and terrestrial radiation on the physical conditions of insectan habitats and on the insects themselves. Prior to the development of the main theme of the paper, certain general points are outlined below.

Researches in ecological climatology with few exceptions have been directed towards analyses and assessments of the large and intensely localized differences in climates occurring within various types of cover or in areas of varying relief. Many investigations in this broad field have provided information of value to both biologists and meteorologists, but the somewhat standardized methods adopted have tended to minimize their value when they are applied to some entomological problems. Thus, the climate of the air surrounding a plant may be of very direct importance to that plant, but it does not necessarily follow that the same climate must have a continuously direct effect upon insects living upon or within the tissues of the plant. Therefore, there are times when data gathered by the usual methods may be definitely misleading.

Similar objections have been raised in the past (21, 22) and, in fact, led to a movement to study the microclimates of the actual dwelling places of insects. However, a subsequent broadening of the concept of microclimate has given rise to considerable confusion and, presumably, to the gaps noted between several laboratory analyses and attempts to apply them under field conditions. Under the heading of "microclimate", recent ecological literature appears to include everything from the humidity gradient above a transpiring leaf to observed variations of temperature among hill and valley stations, with the bulk of the investigations approaching the latter extreme. The impact of this new "microclimatology" upon entomology has led to adoption of some of its less precise techniques without serious consideration of the purposes for which they were originally designed.

Among entomologists, there is still a general tendency to assume that the temperatures of the various parts of plants, particularly the leaves, differ little from ambient temperatures. Yet, Curtis (5) has pointed out that plant surfaces during calm nights must be colder than the surrounding air; otherwise observed dew or frost deposition upon them would be hindered when air temperatures are above the dew point. Also, it was demonstrated (3, 4) that leaf temperatures rise well above those of the ambient air under the influence of solar radiation. This has been known to plant physiologists since about 1909 (for earlier references, see citations above), although at times there has been some disagreement among observers (cf. 24) as to the validity of the differences noted. Curtis (5) has covered these points adequately, and his arguments have the advantage of resting upon a sound physical foundation.

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Plant physiologists have been concerned with this problem chiefly because of the possible influence of leaf temperatures upon transpiration. Nevertheless, examination of their data by an entomologist immediately raises the question of how such differences in temperature affect phytophagous insects during their development upon or within the plant tissues. Should these differences be considered in problems in which temperature is a factor, or are they small enough to be ignored and rejected in favor of thermograph temperatures? Since daytime differences measured by Curtis at times amounted to more than 10 Centigrade degrees, it would seem necessary to take them into account if they can be shown to occur in habitats of insects. Since many economically important insects feed exposed upon the surfaces of leaves, roll or web leaves, or mine within them or within vegetative buds, expanding shoots or fruits, the whole question seems worthy of serious consideration.

There is another point meriting consideration. Although it is not directly stated in standard texts, and despite some good evidence to the contrary (e.g. 9, 13, 23), it is frequently assumed that insects have body temperatures differing negligibly or not at all from those of their surroundings. Although this occasionally may be true under certain laboratory conditions, it seems to be an unwarranted assumption under a great many natural conditions. This and the previous points form the bases for the development of the present account.

LOCATION

Observations have been made at and near Sault Ste. Marie, Ontario. There is a possibility that some of the phenomena observed may be, in a quantitative sense, a function of latitude. Therefore, the reference line adopted is latitude 46° 30′ North. A tree in the grounds of the Forest Insect Laboratory was used as a point for winter observations; spring and summer observations were made at the Pointe aux Pins Insectary, the grounds of which contain disturbed second growth, in which *Pinus resinosa* Ait. and *P. Banksiana* Lamb. are co-dominant species, *Abies balsamea* Mill. and *Acer rubrum* L. are intermediate and suppressed species, and *Populus tremuloides* Michx. occupies openings among the pines.

EQUIPMENT

Standard "Stevenson" screen equipment was used in conjunction with a rain gauge in the insectary grounds. A barograph was kept in one of the buildings. Records obtained from these instruments were supplemented by visual observations of cloud amounts and types, together with wind velocities recorded by an anemovane-anemograph assembly. These general weather instruments were used to log frontal passages or air mass modifications.

Micrometeorological records were obtained with thermoelectric equipment. Leads of either number 24 or 30 copper-constantan glass-insulated duplex wires were used. Various reference-bath temperatures were employed, depending upon the type of investigation and upon the type of ambient conditions encountered. Thermojunctions were calibrated against each reference temperature. For field use at a permanent installation, an ice bath has little value. In some thermometric work (2), it had been found useful to immerse the cold junction box in a bath of transformer oil contained in the well of the bulb of a mercury thermoregulator. This, when used in a larger bath, provided a steady reference temperature near room level. On the basis of this idea, it was decided to use a reference bath heated to a temperature above any which might be encountered in the field and attach the reference junction to a regulator bulb in much the same way as that noted above. Apart from providing a reference temperature which was steady and quite independent of field conditions, this also had the advantage that all temperatures encountered were on one side of the reference temperature, an important item in continuous recording. Since all temperatures were below that of the reference, it was necessary to reverse the polarity of the thermocouples to obtain records.

The bath currently in use was constructed by J. M. Cameron to meet the above specifications. Glycerine was used as the bath fluid, to avoid undue losses, and a steady reference temperature of 58.4° C. was obtained by using a "Chromel" coil heater enclosed in a glass tube, and a mercury switch, which operated the heater through a relay. This equipment has given excellent service. Since early 1948 no additional glycerine has been required, and, apart from occasional chatter in the relay, which can be readjusted, no servicing has been required.

Single-junction couples have been used in preference to thermopiles, since the latter are too much affected by radiant energy. Satisfactory sensitivity was obtained by modifying the meters noted in a succeeding paragraph. Both individual and multiple sets of leads have been used. When multiple circuits were employed, they were arranged to work through a single reference junction, and this arrangement has given no trouble to date. Paired wet and dry thermocouples (cf. 15, 18, 20) have been used on occasion to determine atmospheric moisture levels in restricted spaces having little or no air movement.

Two types of potentiometers were used. A portable, dual-range potentiometer was used for "spot" indications in conjunction with either an internal or a highly sensitive external galvanometer, as the occasion demanded, and a multiple-point, recording potentiometer was employed to obtain 24-hour traces of the fluctuations of several variables on a single chart. A maximum number of 12 different leads could be employed with this instrument, with one junction being recorded every 30 seconds. Thus, although a truly continuous trace was not obtained for individual junctions, a variable at any point could be recorded every 6 minutes, when all contacts were in use, or more frequently, by short-circuiting some contacts, when fewer than the set of 12 were needed. This instrument was modified by resistor changes to reduce its full-scale reading from 15 to 3 millivolts. This gave a very open scale with single-junction leads.

A thermoelectric pyrheliometer was used to obtain records of radiation during daylight. When the instrument was attached to a recording potentiometer, continuous records of the fluctuations of radiation under cloud covers of varying densities were obtained. In addition, simple apparatus for recording temperatures similar to those found on some plant surfaces was employed in 1948 and 1949. This has been noted elsewhere (27). The thermograph employed for this project can also be combined with another, on the black-and-white element principle (12), to record nocturnal cloud, if such measurements are required.

METHODS AND MATERIALS

Much of the microclimatic work was directed towards collection of data on the "surface" temperatures of various parts of trees. A comment on "surface" temperature is necessary at this point. Measurements of the true temperature of any surface cannot be obtained thermoelectrically, since the junction is affected both by the temperature of the surface of the object and by that of the surrounding air. Apart from these factors, introduction of the junction into the system has a rather undesirable influence. Thus, unqualified references to a "surface" temperature are to be shunned. Nevertheless, the measurements obtained do give an approximation of conditions both at and near the surface of an object, as opposed to conditions in the air outside the boundary layer about it, and, if they are accepted in this light, they are very useful for comparative purposes. It is in this sense that "surface" temperatures are discussed in the following pages.

When surface temperatures of foliage were to be recorded, junctions were attached in several ways, depending on the type of measurement required. Measurements of the temperature of an aspen leaf, for instance, could be obtained by threading the wire through the leaf so that the junction was in contact with the desired point on the surface. Measurements of the surface temperatures of twigs or of the needle bases of conifers were made by running the lead along the long axis of the twig to the point desired, and tying it at two points well back from the junction with short lengths of number 30 resistance wire. The junction was then pressed into position in contact with the surface. Measurements of the temperatures of the interiors of needles, vegetative buds or staminate flowers were made in the same way, the free junction being inserted into the part of the tree to be measured.

Measurements of the temperatures of the interiors of buds or needles also are subject to errors, which, in this instance, stem partly from temperature differences in the leads near the junctions. Although such errors may be avoided or minimized if the object to be measured is large enough (6), they are difficult to eliminate when very small objects are being investigated. Once more, the comparative value of such observations must be stressed over the degree of accuracy obtainable. In short, if two temperatures are being recorded in different objects by matched thermocouples under the same ambient conditions, and large differences in temperature are observed, the fact that an actual error of 0.5 Centigrade degrees is operating in the same direction in both readings is relatively unimportant.

Errors of thermocouples due to radiation are more common than is generally admitted, and are difficult to eliminate. The best approximations to actual temperatures may be obtained with the smallest wire diameters practical for the problem at hand, and with the use of suitable shielding. In the present work, the leads were shielded from undue effects of radiation by wrapping them with aluminum foil for a distance of 10 cm. back from the junctions. Air temperature junctions were further shielded from direct sunlight and from the night sky by 5 cm. squares of foil mounted 10 cm. above the junctions. If all junctions are matched to uncover possible differences stemming from faulty contacts or other circuit defects prior to their use in the field, radiation errors may be assessed. If doubts arise as to the validity of foliage temperatures taken with exposed couples, it is best to expose a similar couple nearby to record unshielded air temperature, and to compare both readings with those taken by a shielded air temperature junction at the same level and orientation. Sometimes it may be advisable to make a further check by taking the air temperature at the level with the dry bulb of a calibrated sling psychrometer.

Such checks uncover effects of radiation. If the supposed foliage temperature measured by the first junction was largely a result of radiant heating or cooling of the junction, the unshielded air couple should register very similar results. Both readings should fluctuate quite consistently together and be out of phase with fluctuations of the air temperature measured by the shielded junction. On the other hand, if the greater portion of the reading given by the foliage junction actually resulted from energy communicated by the foliage, a difference between the foliage and air junctions will be noted, and the fluctuations of the exposed air junction will approximate those of the shielded one. This, incidentally, should not be taken to mean that temperatures of air and the objects surrounded by it should never fluctuate in the same direction when the objects are influenced by radiation. Such fluctuations do occur. The point made above refers specifically to the testing of direct effects of radiation on the thermocouples, and independent short-period fluctuations, if at all persistent, are good indications of a reasonable lack of such influence. In the present work, direct influences of radiation on the junctions and nearby leads were reduced to 0.2 Centigrade degrees.

When junctions are attached to portions of a tree in order to compare its foliage temperatures with those of another species, it is necessary to keep both sets of junctions as close to the same level as possible, and also it is desirable to attach them to points having the same orientation. Since it is sometimes impossible to fulfil both these requirements under field conditions, slight positional differences at times tend to obscure relationships. Interpretation of records, therefore, first calls for comparisons of the daily curves from which differences which occur consistently at fixed times may be identified. If these are found, the orientations of points in the installation must be checked and movements of surrounding shadow patterns must be determined. A final check of this type is always necessary before a temporary installation is destroyed or moved elsewhere.

Preliminary observations have been made on specimens of aspen (*Populus tremuloides*), balsam fir (*Abies balsamea*), jack pine (*Pinus Banksiana*), white spruce (*Picea glauca* Voss) and on a specimen of blue spruce

(*P. pungens* Engelm.). Although this list is far from complete, the data accumulated, when considered in relation to previous work (10, 14, 16) provide good bases for more intensive investigations. As noted previously, surface temperatures of foliage and of the bark of twigs have been observed, together with internal temperatures of vegètative buds, expanding shoots, staminate flowers, leaf rolls constructed by a tortricid on aspen and webbed tunnels of the spruce budworm, *Choristoneura fumiferana* (Clem.), fashioned in expanding shoots of balsam fir. In connection with other work directly concerned with the spruce budworm (26), body temperatures of larvae of the final stage were measured in relation to tunnel temperatures and air temperatures, and some of these data are incorporated as illustrative material. In addition, some measurements of the web temperatures of tent caterpillars, *Malacosoma* spp., and of the fall webworm, *Hyphantria textor* Harr., have been obtained during an investigation still in progress, and some examples of these are included.

RESULTS

The observations reported below have been arranged to follow a seasonal trend as closely as possible, in order to demonstrate degrees of differences of microclimates peculiar to definite periods of the year at higher latitudes. Where possible, variations stemming from conditions associated with daylight and darkness and from the presence or absence of cloud or precipitation are noted. It was thought best to present as much of the data as possible in the form of extreme differences rather than in the form of lengthy tables. As may be seen from the few illustrations included, the extremes listed are not merely momentary occurrences but persist over lengthy intervals.

Winter

The first series of observations of entomological interest was complied during the winter. The series was begun to investigate the possible importance of tree-borne snow to stages of various insects overwintering upon or within the twigs of trees. Although the moderating influence of ground-surface snow cover upon the temperatures of underlying soil is well known, and its significance to insects overwintering there has been pointed out (17), the effects of snow attached to the branches of trees upon the surface temperatures of those branches has been an open question. On theoretical grounds, the snow should exert a moderating influence, by virtue of its powers of reflection during sunlight and its properties of insulation at all times, just as it does on the ground. Nevertheless, examination of snow-covered conifers shows that, while snow on the upper surfaces of branches might accumulate to depths of 20 cm. and remain in place under sheltered forest conditions, penetration of the snow through the spaces between twigs is poor, usually amounting to less than 3 cm. Thus, except when two snow-laden branches become fused within a common mantle of snow, the under-surface of a branch would appear to be poorly insulated and subject to penetration by cold air currents. Direct measurements were required to clarify the situation.

Thermojunctions were attached to twigs of an isolated Colorado spruce standing on the snow-covered lawn about 8 metres to the south of the laboratory. A more rigorous test of the efficiency of snow cover could scarcely have been devised, since the tree was exposed to direct sunlight and stood in a wind channel some 25 metres wide, directly in the path of the prevailing winter winds. Junctions were applied directly to the upper surfaces of the bark of terminal twigs, so that measurements of temperature at needle bases could be obtained. One twig was kept free of snow, while another at the same level and approximately the same orientation was kept covered with 8 to 10 cm. of snow, the lower surface of which finally adhered to the needles and protruded 1 cm. beneath them, the whole remaining in place even at relatively high wind velocities. Air temperature at the same level was recorded by a separate junction midway between the two "foliage" junctions. Both potentiometers were used at different times to obtain records. The results are summarized below.

During the daylight hours, the snow cover on the branch reflected some solar radiation. With air temperatures ranging from −15 to 0° C., the temperatures of the snow-covered foliage ranged 0.5 to 3.5° below the air temperatures under clear skies, broken cloud or thin overcast, with the larger differences occurring during the hours of maximum insolation. Strong winds did not appear to diminish the differences appreciably. No indication of undue absorption of radiation by the junction through the snow (cf. 8) was noticed, possibly because the lead diameters were smaller than those ordinarily used in snow temperature measurements.

By day, the exposed foliage usually exhibited temperatures in excess of those of the air, indicating a marked amount of radiant heating even at the low solar altitudes of winter. Maximum elevations above the ambient temperature which were noted in clear weather approached 2.2°. Thus the exposed foliage was, on occasion, over 5° above the snow-covered foliage. These differences in temperature were subject to rapid fluctuations, but, in sunlight, the variations were never accompanied by a change in the sign of the difference. The exposed foliage was warmest, with the air at an intermediate temperature and the snow-covered foliage at the lowest temperature. Under thin cloud, sufficient radiation penetrated to maintain the exposed foliage 1° or less above air temperature. Under dense cloud, the difference was negligible. When an isolated cloud obscured the sun, but left the overhead sky clear, radiation losses lowered the temperature of the exposed foliage 1° or less below the air temperature. It is interesting to note that with winds of 28 to 33 m.p.h. (13.9–17.1 mps.), the exposed foliage was still warmed 1–2° above the ambient temperatures by radiant heating.

The reverse situation occurred by night. Under nocturnal conditions within the polar continental air masses common to the interior of North America in winter, the surface of the earth and the exposed objects upon it radiate freely to the upper air. The surfaces follow approximately the laws of black-body radiation, and since their temperatures, however low, are warmer than those to which they radiate, surface temperatures fall well below the temperature of the air immediately surrounding them. Under such conditions, air temperatures near the surface follow the surface temperature, largely by conduction and extremely slow convection, rather than the exposed surface taking up the temperature of the air, as at first thought one might expect. The net result, if measured as air temperature, is revealed in the presence of a surface inversion, within which air temperature increases rather than decreases with height (25).

This situation proved to be of importance to the results being described. Under clear skies, and with little air movement, the snow-covered twig remained warmer than either the air or the exposed foliage, since its snow cover insulated it rather effectively from direct conduction from the surface layer of air and also, to a certain extent, retarded its radiation. Although radiation took place, its rate was slower than that from the surface of the exposed foliage, which, because of its loss of heat by outgoing radiation, cooled below the ambient air temperature. Under the above conditions, snow-covered foliage was 0.5° warmer than air at −20° C., or 1.7° warmer than air at −15° C., whereas, at the same times, exposed foliage was 0.3 to 0.8° cooler than the air, or 0.8 to 2.5° cooler than the snow-covered foliage. The ranges noted above are small, but indications are that they might have been increased in a more sheltered situation. The wind channel mentioned previously proved to be particularly troublesome at night, when it became part of a local situation which was meteorologically interesting, but a hindrance to attempts to determine effects of radiation on the temperature of various surfaces.

On "good" radiation nights, a street down a low hill to the north of the laboratory acted as a channel through which air chilled by radiation on the hill top moved in much the same manner as katabatic winds move down slopes or funnel along valleys at night. Buildings along the street channeled the air, and, towards midnight, a 7 to 10 m.p.h. wind (3.4-5.4 mps.) had developed which entered the wind channel in which the tree stood, thus breaking down the radiation pattern in that area. This occurred even on nights when strong inversions were forming in other parts of the town, as evidenced by the layering of chimney smoke at moderate elevations. Yet, although troublesome, this wind did show the destructive effects of even slight air movement upon nocturnal radiation proceeding at a low temperature level.

In experiments upon the relation of leaf temperatures to transpiration (3), it has been mentioned that leaves radiate to temperatures below that of the air even when strong winds are present. As it stands, this statement is incomplete. It should include a notation to the effect that leaves do so *if* their temperature level is high enough. According to the Stefan-Boltzmann law, the rate of radiation decreases when the temperature of the body decreases. Thus, a wind speed which is not sufficient to disrupt completely radiation from a leaf surface at high summer temperatures will be more than adequate for the purpose at low winter temperatures, because the rate of radiation is considerably less at the lower temperatures.

Accordingly, wind speeds of about 2–3 mps. were sufficient to break down the radiation patterns at the temperatures of −12° C. or less which were common during the observational nights. Since these winds, although intermittent, occurred frequently, inspection of the data suggests that maximum differences between the temperatures of the air and the exposed foliage were seldom, if ever, attained. Nevertheless, the differences which were observed did indicate real variations in the rates of radiation at different temperature levels. The difference of 0.3° was recorded in the air temperature range of −20 to −23° C., whereas the 0.8° difference was observed in the range from −15 to −18° C. Calculation of the percentage decrease of flux per Centigrade degree decrease of temperature shows a continuous decrease in the rate of heat loss by radiation over the range from +60 to −60° C. and indicates the interesting possibility that, at temperature levels below approximately −30° C., which are not at all unusual in the northern forest region, the difference between the temperatures of exposed foliage and its surrounding air might be negligible for practical purposes, even when undisrupted radiation is in progress.

Although wind disrupted differences observed between air and exposed foliage, and tended to eliminate them, it also exposed the chief weakness of tree-borne snow cover on an isolated branch. The lack of a sufficiently thick insulating layer on the under surface of a branch permitted penetration by turbulent currents, so that the temperature of the snow-covered twig also approximated that of the air at moderate wind velocities, being lowered by the penetrating air.

The presence of nocturnal cloud changed the relationships described above according to the density and uniformity of the cloud cover. Thin, high stratiform cloud exerted a negligible influence, diminishing differences by a barely observable amount, and broken cloud exerted little more influence. On the other hand, an overcast of low, dense stratiform or cumuliform clouds reversed the sign of the difference between temperatures of exposed foliage and the air. Under such conditions, even with the low prevailing temperatures, the exposed foliage absorbed sufficient quantities of reflected and re-emitted radiation coming from the clouds to have its temperature raised up to 0.3° above that of the surrounding air. Lifting of the cloud base or thinning of the cloud was characterized by an immediate decrease in this difference. The foliage temperature once more approached that of the air, and, with clearing skies, dropped below it again. Snow-covered foliage usually was maintained at a temperature about 0.5° above the air under an overcast.

The results described above indicate the moderating influence of tree-borne snow cover upon the temperatures of underlying twigs and foliage. Since the differences are presented in Centigrade degrees, they appear to be rather small, and, in comparison with temperature differences existing between the top and bottom of a layer of snow on the ground, they are. Nevertheless, viewed from the standpoint of the wide fluctuations in environmental temperature experienced by an insect overwintering on a tree, the differences may be important to survival. Translated into terms of the daily march of temperature, they mean that an insect on a branch covered by a light layer of snow would, while air temperature moved through a 23° range in a day and a night, be subjected to temperature fluctuations ranging through 17 or 18°. An insect on a fully exposed branch would be subjected to fluctuations through a 26° range during the same period. When generally low temperatures prevailed, the stress imposed upon the ability of the exposed insect to acclimate rapidly, or upon its ability to withstand temperatures near its lower lethal range, might be of considerable importance to its ultimate survival.

It should be remembered that the foregoing observations were made on a tree having the maximum exposure to winds. The effects of surrounding trees upon the retention of snow cover by conifer branches is very great, and field observations indicate that all but the most exposed portions of the crowns retain the bulk of freshly fallen snow during most of the period of cold, clear weather characteristic of winter polar air masses interjected between periods of snow-fall, so that, except during actual thaws, the supply of snow in the crowns of a moderately dense stand is maintained and replenished. Thus, the vertical distribution of a population over-wintering on a tree becomes of importance, if any statements based on laboratory determinations of lethal temperature ranges are to be extended to cover field conditions.

Snow retention by the branches of deciduous trees is much less than in conifers. This appears to be a result not so much of the lack of surface area for snow retention but of the greater reaction of a whole tree to wind action upon its top, if this is at all exposed to wind. Vibration and shearing in such circumstances account for a greater portion of the snow lost. It has been noticed that smaller deciduous trees partially overtopped by other trees retain a light snow cover along the upper surfaces of the branches, and frequently retain balls of snow 20 cm. thick in the regions of the forks. The type of snow is also important where deciduous trees are concerned. During the winter of 1947-48, much of the snow which fell in the vicinity of Sault Ste. Marie was powder snow, which was scarcely retained by deciduous trees. During the winter of 1948–49, the majority of the snow types consisted of stellar crystals, hexagonal plates or graupel forms (19) and falls of these types remained on main branches which did not retain powder snow.

In closing this sub-section, it may be noted that in lower latitudes, with milder winters and higher values of insolation, daytime discrepancies between air and tree temperatures will be increased considerably. For instance, a recent research note (1) gives differences of about 26° in favour of cambial temperatures on the sunlit side of a tree at air temperatures near 7° C.

In connection with insects themselves, rather than with their over-wintering sites, the only available material on which direct measurements could be made has been egg masses of the forest tent caterpillar, *Malacosoma disstria* Hbn. During February, 1949, records were taken of the temperature on the upper side of a mass, just beneath the protective layer of material covering the eggs. A specimen set of observations is shown in Table 1. The measuring equipment had no apparent ill effects upon the eggs, for they hatched outdoors in late April, 1949.

Spring and Summer

The following descriptions are applicable to the period covered by the months, May through August. In the first sub-section on general trends, results are presented in summary form only, by citation of extremes noted, as in the bulk of the descriptions of winter events.

General Trends

Diurnal Observations

Clear Sky

During daytime in the first week of May, surface temperature of blue spruce foliage of the previous year's growth was raised 2.8 to 5.6° above the temperature of the surrounding air, when the air temperature ranged between 12.8 and 15° C. When the foliage was shaded from direct sunlight, but remained exposed to a clear sky, its temperature ranged from 0.8° above to 2.2° below the air temperature. The intervals during which the shaded foliage was above the air temperature were very brief, coinciding with movements of the over-topping foliage which permitted some penetration of solar radiation.

In the direct sunlight of late May, the surface temperatures of the needles and expanding vegetative parts of the coniferous species tested tended to be very similar to one another under conditions of uniform elevation and orientation. Moreover, the temperature of air enclosed by an aspen leaf rolled by a tortricid larva also approximated the temperature of the types of foliage noted above. On the other hand, normal aspen leaves had temperatures which were markedly lower than the other temperatures observed. All foliage temperatures were above ambient ones in sunlight.

The difference between normal aspen leaves in sunlight and the needles or vegetative buds of the coniferous species was apparent in all records examined. At air temperatures between 15 and 26° C., aspen leaves

TABLE 1.—INTERNAL TEMPERATURES, IN CENTIGRADE, OF AN EGG MASS OF *Malacosoma disstria* HBN. DURING A TWENTY-FOUR HOUR PERIOD

Time: EST.	Air temp.	Δ Egg temp. − air temp.	Weather notes
0000	−11.5	0.2	Moderate snow; wind 15-20 m.p.h.
0100	−12.3	0.1	Clearing; wind dropped to near calm
0200	−11.1	0.0	
0300	−12.3	−0.3	
0400	−13.4	−1.0	
0500	−14.6	0.2	Cloud patch
0600	−14.8	−0.7	
0700	−15.6	−1.0	Greatest negative Δ at 0630, −1.3
0800	−15.5	−0.1	Isolated stratiform patches
0900	−14.4	−0.9	Cloudless; hazy (eggs shaded)
1000	−10.6	0.0	
1100	−12.0	1.6	
1200	−11.0	3.4	
1300	− 9.0	4.9	Stratiform cloud on horizon; largest positive Δ, 5.3 at 1330
1400	− 8.6	−0.5	
1500	− 8.5	1.9	
1600	—	—	
1700	− 7.9	0.4	Overcast altostratus from 1530
1800	− 8.2	0.0	Overcast altostratus
1900	− 8.8	−0.2	Overcast, thinning
2000	− 9.5	−0.1	
2100	− 9.5	0.0	Overcast, thickening
2200	− 8.7	−0.2	Overcast
2300	− 7.3	−0.1	Overcast
2400	− 6.0	−0.1	Overcast

ranged only 0.5 to 1.6° above the air, whereas coniferous foliage ranged from 1.6 to more than 8° above the air, usually being maintained at least 5° above air temperature. Closer examination of the situation indicated that this discrepancy between the broad-leaved tree and coniferous species might result not so much from the continuous movement of the aspen leaves, since whole conifer branches were often in motion during the same periods, as from the angle at which the deciduous leaves were presented to the rays of the sun. All conifers, even jack pine, presented surfaces more closely perpendicular to incoming radiation than did aspen. It may be doubted that the angle of incidence could have such a profound effect, but further evidence exists in data obtained in the aspen leaf-roll. Tortricid larvae drew pairs of aspen leaves together in such a way that each pair projected outward at an angle of about 40°, instead of hanging almost vertically as did normal leaves. As noted above, the temperature of the air enclosed by these rolls approximated that of the surfaces or the interiors of coniferous foliage.

During the summer, surface temperatures of coniferous foliage exposed to direct sunlight moved through approximately the same range above air temperatures as was noted in the preceding paragraph, although there was some tendency for average values to remain a fraction of a degree higher than those noted for late May. On the other hand, when the foliage was shaded from direct sunlight, but remained exposed to the sky, its temperature dropped 1 to 3° below the air temperature, when this was in the vicinity of 30° C., as opposed to the maximum drop of 2.2° noted for spruce in air at 15° C. Strong winds exerted no readily observable influence upon the foliage or bud temperatures during hours of sunlight.

Clouded Sky

Introduction of clouds into the system produced a number of complications in the foliage-air temperature daylight relationships. Seasonal differences in cloud structure related to seasonal trends in air mass types also exerted recognizable influences upon the relationships.

During early May, thin, stratiform clouds, such as cirrostratus, or broken clouds, such as altocumulus, were high enough and thin enough to allow the passage of enough insolation to maintain fully exposed foliage 2.8 to 3.9° above air approximating 15° C.; a reduction of only 1.7° in the maximum elevation of temperature under clear sky. During the whole month of May, scattered cumulus clouds over the area were a turbulence type of fair-weather cumulus (Figure 1) and were neither slow enough nor sufficiently developed vertically to exert any appreciable influence. Under a broken sky, foliage which was shielded by other foliage from the sun but not from the sky received sufficient cloud-reflected radiation to maintain its temperature about 1° above that of the air, in direct contrast to the comparable situation under a clear sky. Under a heavy overcast of either stratiform or cumuliform clouds, all foliage was maintained about 1° above air temperature by diffuse radiation. The latter result differed from the observation under heavy diurnal cloud in winter. The difference resulted from stronger summer radiation.

During the summer months, further information on the relation of cloud types to foliage temperatures was obtained with the pyrheliometer.

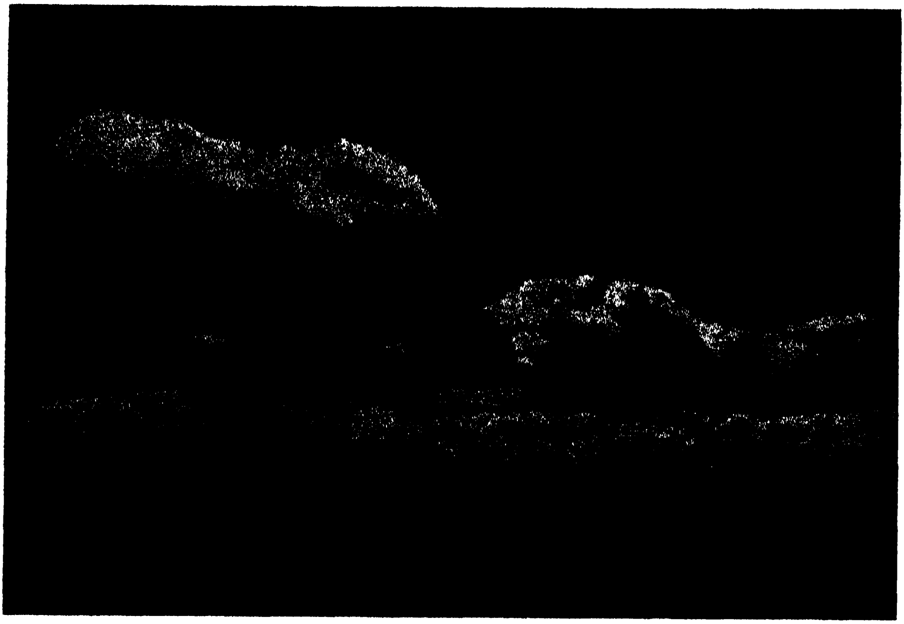

FIGURE 1. Low-level fair weather cumulus, turbulence type, *circa* 500 meters altitude, moving from the west with a surface wind of about 20 m.p.h. (8.9 mps.).

In the records, several examples of sudden, brief reflections from passing individual clouds occurred, which added momentarily to the total radiation value recorded. These "zooms" (cf. 11) are of uncertain meteorological significance, but are definitely of no importance to foliage temperatures, since their periods of occurrence are too brief to bring a response.

As in May, scattered cumulus cloud moved too rapidly to have much influence upon the foliage. Judging by the records, if the period of fluctuation of the intensity of radiation was less than 3 minutes, no observable effect occurred in the foliage temperature. A period of serious depletion of solar energy more than 3 minutes in length, such as occurs when the towering portion of a heavy cumulus passes overhead, resulted in radiant cooling of the foliage, to or below the level of the air temperature. On the other hand, when stratiform cloud appeared on summer mornings, foliage temperatures averaged 2° above air temperature, as compared to 1° in May.

It was observed that, whether the insolation was rising or falling in value, swings superimposed upon the trend gave comparable swings in foliage temperatures. A total swing of 1.1 to 1.3 gm. cal./cm.^{2} gave a swing of about 6° in foliage temperature; 0.7 to 0.9 gm. cal. fluctuations gave a foliage fluctuation of 4°, and 0.2 gm. cal. fluctuations gave swings in foliage temperature amounting to about 1°.

Rain

When rain fell, the differences between the temperatures of the air and the trees were of either sign, but were negligible. On the other hand, as soon as the rain stopped, evaporation from raindrops resting on the foliage lowered its temperature as much as 2.8° below that of the air for as long as 40 minutes.

Nocturnal Observations

Clear Sky

The phenomena observed at night depended partly upon the weather of the afternoon and partly upon the weather during the night. If a clear day was followed by a clear night, during which there was little or no air movement, both the air and the various foliage temperatures fell to low values. In May, air temperatures usually dropped to the vicinity of 0° C. during such nights. Radiant cooling of all types of foliage lowered their temperatures 1 to 1.2° below the air. When the air temperature fell only to 4.5°, the foliage cooled 1.6° below the air.

Fog occurred often between 2200 and 2300h. on clear spring nights. About 10 minutes after fog was noticeable, a sudden drop in temperature occurred which once amounted to almost 4°. However, as the fog thickened, the air temperature rose slowly, presumably because of heat liberated by condensation, and the foliage temperatures gradually rose towards the air temperature, since the fog droplets absorbed and re-radiated most of the original outgoing radiation. If the fog suddenly dissipated, within 10 minutes of its disappearance a second drop in temperature occurred, and radiant cooling of the foliage increased the difference between the temperatures of air and tree once more.

During clear summer nights, when the air was in the neighbourhood of 10° C., if it was calm, all foliage temperatures dropped as much as 3° below the air temperature. Strong inversions of temperature occurred in the lower air on such nights. No upper air moisture data were available, but it was noticed that on other nights which appeared to have no more air movement, radiation decreased the tree temperatures to only 1° below air temperatures, and, in the lowest layers at such times, only weak inversions or isothermal conditions were present. While this occurred often with some air movement, its occasional occurrence under apparently calm conditions suggested the presence of increased water vapour content of the air above the station at such times.

Clouded Sky

If afternoon cloud dissipated before or shortly after sunset, the conditions noted above for a clear night prevailed and the same differences were observed. If clouds persisted into the evening, radiation from nocturnal cloud-types was not sufficient to maintain the foliage temperatures more than 0.1° above the air temperatures during spring and summer. In fact, a dense, stratiform overcast was required to maintain any vestige of equality between air and tree. This was in contrast to the situation observed during winter. Lifting or breaking of night clouds was indicated by a sudden cooling of the foliage until the differences previously indicated were reached.

While foliage differences, such as that noted between normal aspen leaves and coniferous foliage occurred during the day, no such difference was observed at night. Apparently radiation from the trees took place in approximately equal quantities, regardless of species, or of different portions of any one species (see the notes on staminate flowers below). Fluctuations produced differences of less than 1° among foliage types, but, since signs frequently reversed, differences tended to cancel.

Rain

Rain at night had the same effect as it had in the daytime. Differences between air and trees were present, and of either sign, but were negligible.

The foregoing observations contain points of interest from the standpoint of insect ecology. The increased differences observed during the spring indicate that, while a sheltered thermograph might register a 24-hour temperature range of some 15 degrees, insects nearing the end of their period of hibernation upon the twigs of trees may be experiencing a range of 21 or 22°. Furthermore, 5° or more of this range lies at temperatures above the maximum shown by the thermograph. Under summer conditions, when insects are feeding on leaves, this difference is increased, as shown in the notes on summer temperature levels.

The observations on rolled and normal aspen leaves, which suggest the possibility of the importance of angle of incidence, raise a point which is not of direct entomological concern, but is nevertheless interesting. Many specimens of the black spruce (*Picea mariana* BSP.) exhibit typically pendulous branches. Bursting of the buds of this species occurs later than in other species of spruce. Doubtless this delay is largely a result of specific differences, but it would be interesting to examine the possibility of whether or not the angle at which buds are held in spring sunlight by the drooping branches is of importance to the observed discrepancy in phenology. The question might have some bearing on problems centred on the habits of the spruce budworm.

A Frontal Sequence

Figure 2 is a composite illustration showing changes in various meteorological factors over a four-day period in the latter half of August. The upper portion of the figure shows the changes in wind direction and atmospheric pressure associated with the approach and passage of a warm front, which, at the ground, passed over the station area about 0400 on the third day. Note that the wind at night commonly had a northerly component. This periodic variation complicated the picture slightly, but if it is taken into account while the record is examined, the shifts of wind direction associated with the front are clear. The temperature curves illustrated are based on half-hourly abstracts, in order to obtain an intelligible curve at a reduced scale. This method has the disadvantage that many of the independent fluctuations of the plant temperatures are lost.

The internal temperature of a developing cone of *Pinus Banksiana* (solid line) should be compared to the air temperature (dotted line) at the same level, 2m. The broken line shows the fluctuations of soil surface temperature, measured on sand near the base of the tree, and the remaining line shows the temperature of a grass blade 5 mm. above the soil surface point. It is particularly interesting to observe the effects of nocturnal radiation on the grass-blade temperature as compared to the soil surface temperature. The basis for dew formation on turf is clear.

On the first day of the series, broken cumulus occurred until 0900. Thereafter, scattered cumulus persisted until 1400, after which only isolated clouds occurred. By 1700, the sky was completely clear, but in the early evening, remnants of the day's clouds occasionally passed over.

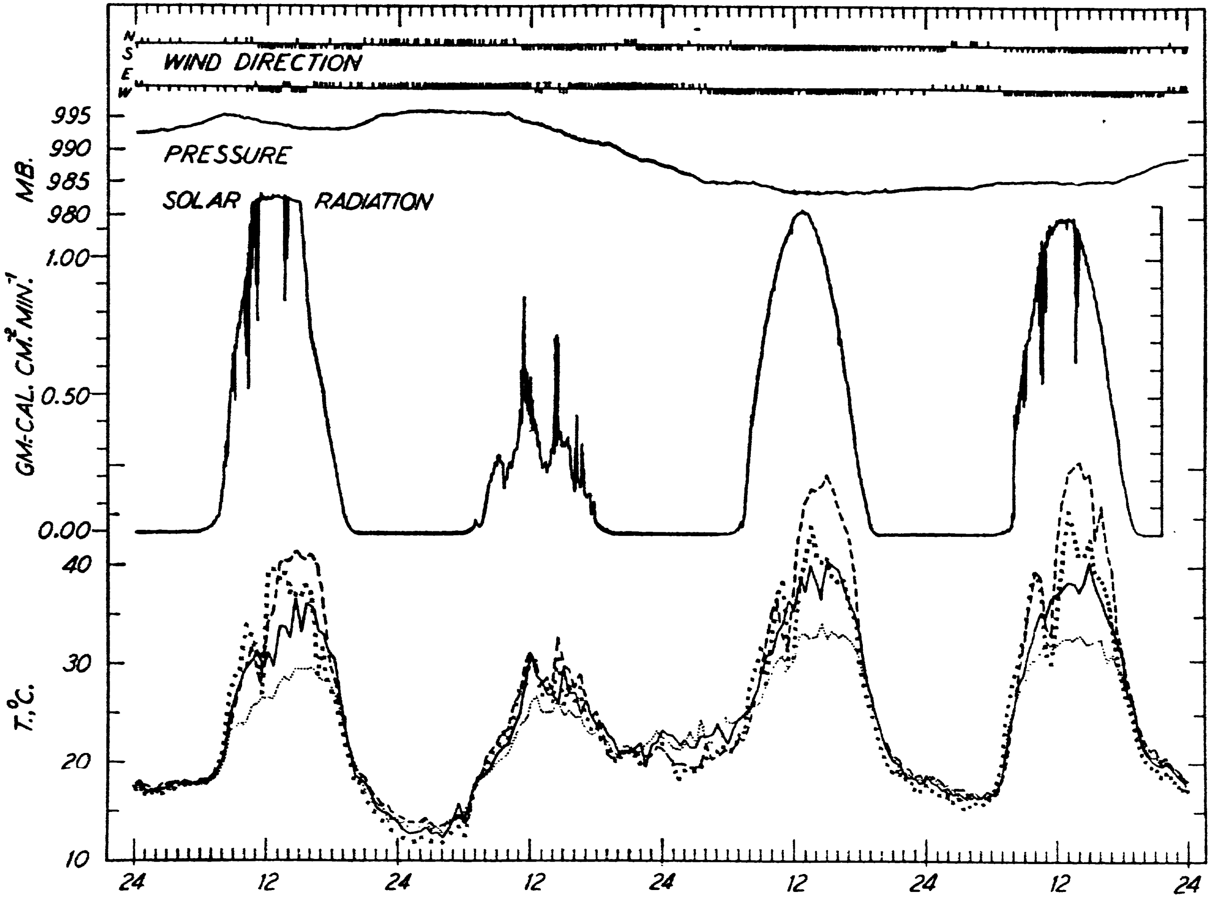

FIGURE 2. Changes in meteorological factors and associated variables over a 4-day period during which a warm front passed over the station on the third day. Abscissa: time, in hours. Ordinate (temperature curves): dotted line, air temperature at 2 m.; solid line, internal temperature of a developing cone of *Pinus Banksiana* at the same level; broken line, sand surface temperature at the base of the tree; crosses, grass blade temperature 5 mm. above the soil surface.

On the second day, an altostratus overcast appeared just after 0700, and, thereafter, the sky was clouded (4–10/10), with altostratus and altocumulus predominating. At 2000, there were 8/10 altocumulus, and the sky was occasionally completely overcast during the night, as also is shown by the temperature curves. Only a trace of rain was found in the gauge the next morning.

The third day was cloudless by 0800, and the sky remained so throughout the day and night, although there was a persistent, heavy haze. The fourth day was characterized by the appearance of a few fair weather cumulus clouds, but remained hazy and hot.

SPECIAL SITUATIONS

The preceding summation of differences observed between the temperatures of buds, shoots or leaf surfaces and that of the surrounding air showed that, particularly during the hours of daylight, large differences exist. In the present section, differences of particular entomological interest or those which have been observed in special situations are described.

Coniferous Staminate Flowers

The flowers of coniferous species exhibited internal temperatures which were considerably higher than ambient temperatures in direct spring sunlight. Figure 3 shows a series of records taken over a 24-hour period. One junction was inserted in a staminate flower of white spruce, another was inserted in a vegetative bud which had burst and was showing green foliage for a total length of 5 mm., while a third junction was installed nearby to measure air temperature. On the day in question, the area was occupied by a polar air mass which had moved over the region during the preceding 24 hours. Post-frontal heavy cumulus had broken during the preceding 12 hours and the air mass had been slightly modified. Nevertheless, air temperatures were still relatively low, despite the presence of a clear sky and bright sunlight. Winds were of the order of 8 to 18 m.p.h. (3.4–7.9 mps) during the day, dropping to 3 m.p.h. (1.5 mps) or less during the night.

The curves shown in the figure were plotted by taking the temperatures at 10-minute intervals and joining the points with straight lines. The method permits retention of the major fluctuations, and illustrates the major differences involved, together with any reversals of trends. The part of the figure illustrating the daylight period shows that the flower was 5 to 8.4° above the temperatures of the vegetative bud, and often ranged 10 to 14.5° above the air temperature. The slight displacement of the maximum of the flower curve into the morning stemmed from the fact that the flower cluster observed was orientated slightly east of south, whereas the vegetative bud was orientated almost due south. The crossing of the temperature curves for flower and bud about 1600 rather than later in the day also was a result of the orientation difference. The significant features shown are the wide discrepancies among the three curves and the length of time these differences were maintained. As a matter of general interest, this figure also shows the displacement of the air temperature

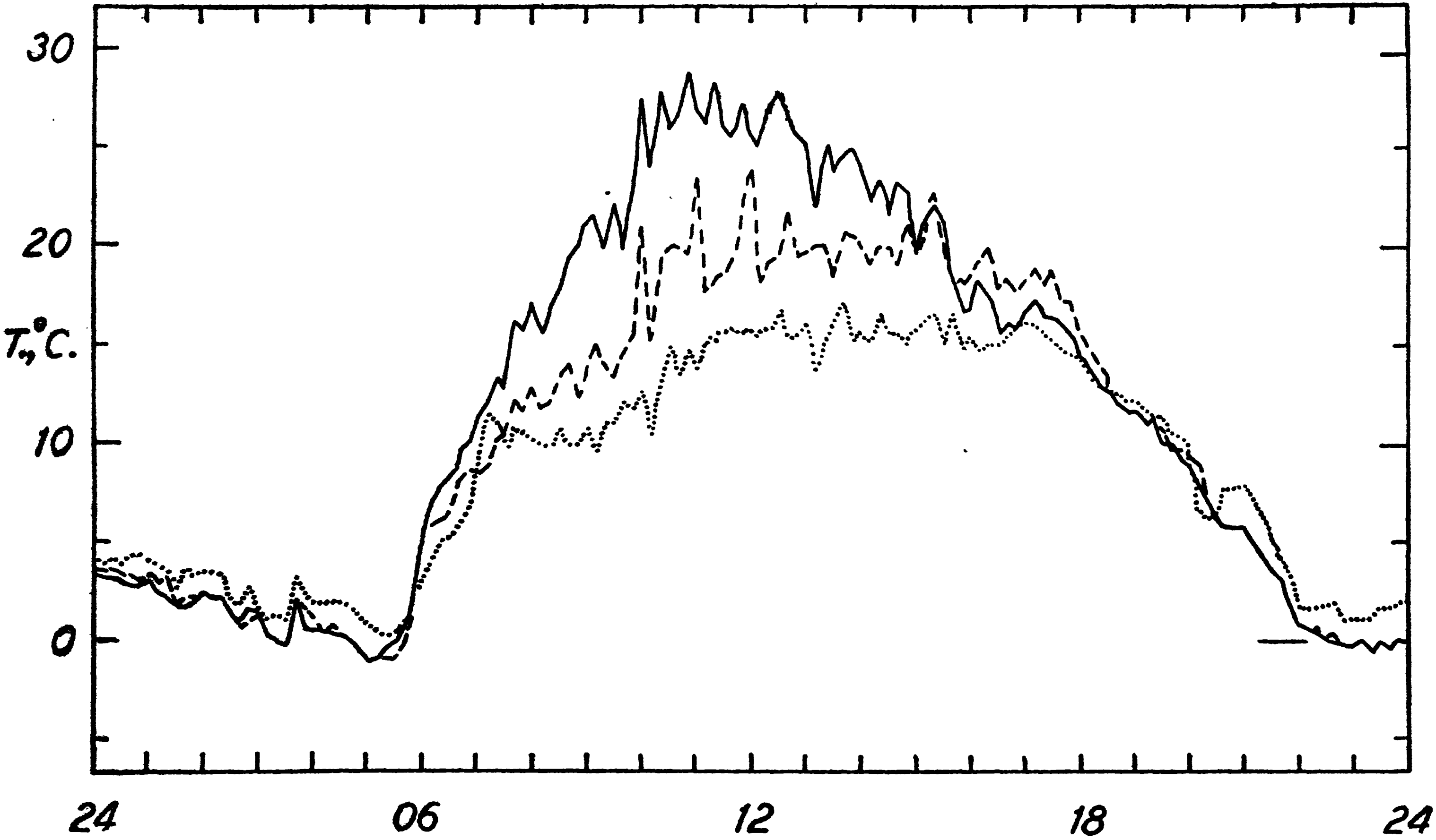

FIGURE 3. Comparison of the internal temperatures of the staminate flowers and vegetative buds of white spruce with the surrounding air temperature within a typical spring polar air mass. Abscissa: time, in hours. Ordinates: Dotted line, air temperature; solid line, flower temperature; broken line, vegetative bud temperature.

maximum towards the mid-afternoon, after some decline of solar radiation, and the maintenance of air temperature at a relatively steady value through the later afternoon, while the tree temperatures, dependent upon immediate insolation, continued to decline.

The immediate entomological significance of the foregoing illustration stems from current interest in the spruce budworm. During the spring, second stage larvae move from their hibernacula on the twigs to their feeding stations at or near the tips of branches. Larvae commonly feed among flowers when these are available. A glance at the accompanying figure shows that staminate flowers provide a much more efficient "greenhouse" when low air temperatures prevail than do vegetative buds. Thus, flowers provide conditions which would tend to accelerate the developmental rate during early instars by permitting steadier feeding. Since the first portion of the feeding stage is a critical period in the development of most lepidopterous larvae, interruptions then being reflected adversely in later stages, the superior "greenhouse" effect of staminate flowers may be of some importance.

During the period of the year when flowers are available to larvae, weather conditions in northern Ontario are characterized by frequent invasions of polar air masses similar to or colder than the one illustrated above. Laboratory tests of balsam staminate flowers indicated that they cooled more slowly than did buds or needles when a source of radiation was cut off, but curves obtained in the field under rapidly moving turbulence cumulus, the typical spring cloud type, showed no special evidence of any advantage gained from the slower cooling rate, because the clouds travelled too rapidly to deplete incoming radiation seriously. With broken, rather than scattered cloud, the slower rate of cooling might operate in favour of budworm larvae feeding in flowers, since it would maintain their environment above the prevailing low air temperatures which are often below 10° C. during cloud passages taking 10 or 12 minutes.

Figure 3 also shows that, for the most part, the flowers and the vegetative bud exhibited no difference in temperature during the night. When nocturnal differences did occur, they were, with two exceptions, in favour of the vegetative bud. This is a further indication that the flowers were closer approximations to a "black body" than was the bud. Nevertheless, differences at night, from the practical standpoint, lack the significance of the greater differences exhibited in sunlight, or the summer nocturnal differences exhibited between air and any foliage temperature.

In the field, balsam fir flowers were not observed under conditions so ideal as those which existed when spruce flowers were measured. A considerable amount of thin cloud occurred during the measuring period. Nevertheless, at air temperatures ranging between 10 and 20° C., daytime records showed flowers ranging 2 to 5.5° above vegetative buds, which, in turn, were 2 to 5° above the surrounding air. Jack pine flowers were inferior to white spruce flowers in sunlight, but they still ranged 2.7 to 5.5° above the bud temperatures.

Spruce Budworm Internal Temperature

Gunn (9) and Uvarov (23) have reviewed work on the effects of radiant heat upon insectan internal temperatures. Previous work under field conditions has, for the most part, been confined largely to investigations of the temperatures of locusts in relation to ambient temperatures. The results suggest that a common source of error in applying laboratory data showing the effects of temperature upon behaviour, development or survival of various insects, has resulted from neglect of radiation in the field. During the study of reactions of the larvae of the spruce budworm to light (26) it was found necessary to measure the body temperature of sixth stage larvae under natural conditions. This work was extended somewhat for the present purposes, and some of the data are included here, since they show that a lepidopterous form is affected similarly to locusts.

To measure the internal temperatures of a larva under field conditions, a thermojunction was inserted into the digestive tract through the anus so that the junction rested in the vicinity of the mid-gut. Table 2 gives sample hourly readings, expressed as differences from ambient temperatures, of two of a number of living larvae which were exposed to atmospheric conditions for 24 hours in late June.

TABLE 2.—INTERNAL TEMPERATURES, IN CENTIGRADE, OF TWO SIXTH STAGE LARVAE OF *Choristoneura fumiferana* (CLEM.) DURING A TWENTY-FOUR HOUR PERIOD

Time: EST.	Air temperature	Larval temperature —	Air temperature
		$\Delta L_1T - AT$	$\Delta L_2T - AT$
0000	3.4	−0.1	−0.5
0100	2.0	0.1	−0.2
0200	1.7	−0.1	−0.5
0300	1.8	−0.5	−0.6
0400	1.7	0.0	0.0
0500	2.5	0.4	0.1
0600	6.9	0.2	0.0
0700	11.5	1.7	1.6
0800	16.5	1.5	2.7
0900	20.0	2.2	2.2
1000	21.6	2.9	1.8
1100	23.7	3.0	1.7
1200	28.2	0.5	−0.2
1300	29.6	−0.2	0.5
1400	27.5	−0.1	0.2
1500	26.4	0.7	0.5
1600	26.2	1.1	0.8
1700	28.2	0.1	−0.1
1800	25.7	0.5	0.9
1900	21.7	−0.2	−0.5
2000	16.0	−0.1	−0.3
2100	12.5	−0.5	−1.4
2200	10.0	−0.1	−0.4
2300	9.2	0.7	0.1
2400	9.0	−0.5	−0.2

The weather during the period was as follows: the preceding evening had been partly cloudy, and the cloud was dissipating near midnight. Scattered patches came overhead occasionally, and some of these occurred at the hour points given (e.g., -0400). Thereafter, the sky was cloudless, but hazy, until after 0800. Scattered patches of altocumulus and cirrus appeared at 0900, and continued to move over the station throughout the rest of the period, which accounts for the lowered temperatures or the reversals of sign near midday and through the afternoon. Slight discrepancies between the two larvae were a combination of individual variation and differences in overlying shadow pattern. The most important point shown is that when the larvae were fully exposed to the atmosphere, their internal temperatures during sunlit periods were quite consistently above the surrounding air temperature, and during the brief periods of radiant cooling at night, fell slightly below ambient temperature. The highest elevation above air temperature during the day occurred at 1230, when the internal temperature of larva 1 was 3.9° above the air.

There may be some objection to the observations described above, on the grounds that sixth instar budworms normally feed in loosely-constructed shelters which they fashion by webbing together some of the needles of a shoot. One reason the above readings were taken in the manner described was that continuous records throughout a twenty-four hour period were required, and it was impossible to make such readings in a budworm tunnel for that length of time, since the silk allowed a larva sufficient traction to pull itself free of the junction after a short time. However, to meet any objection, some readings were taken for short intervals of larval internal temperatures within the natural tunnels. At the same time, the temperatures of the air enclosed in the tunnel and the outside air were recorded, together with the moisture contents. Readings were taken in early July, when somewhat higher temperatures than those previously noted were prevalent. Some of the pertinent records are listed in Table 3.

This table lists the internal temperatures, expressed as differences from air temperature, of some of the sixth stage larvae tested. During the observations, the sky remained cloudless, but there were occasional periods with light haze. Flecked shadow from overtopping foliage approached the observational point near the 1410 reading, and, thereafter, the flecked shadow slowly changed to an apparently continuous one. The differences listed are large and require little additional comment, but it should be noted that some of the negative values resulted from sudden upsurges in air temperature to points above the temperatures inside the tunnels. The wind remained close to 15 m.p.h., with gusts, and the upsurges resulted from sudden passages of warm currents.

Although the primary concern here is with the effects of radiation, it is of interest to note that the tabular data give some indication of the effects of atmospheric moisture upon the internal temperatures of the insects. Furthermore, with the exceptions of the tunnels of larvae 1 and 4, which were very loosely webbed, the humidities listed, when converted into units of evaporation (28), closely approximate the evaporation preference determined for exposed larvae of the same stage in earlier laboratory work (29).

TABLE 3.—INTERNAL TEMPERATURES, IN CENTIGRADE, OF SIXTH STAGE LARVAE OF *Choristoneura fumiferana* (CLEM.) CONCEALED IN FEEDING TUNNELS, TOGETHER WITH TEMPERATURES AND RELATIVE HUMIDITIES OF AIR INSIDE AND OUTSIDE THE TUNNELS

Larva No.	Time: EST.	Air temp.	Tunnel temp. — air temp.	Larval temp. — air temp.	Air R.H.	Tunnel R.H.
1	1143	29.3	0.2	1.0	54.0	96.0
	1145	30.6	−1.6	−0.7	49.0	89.0
	1147	26.7	1.0	2.1	60.0	83.5
	1151	25.8	0.7	2.3	64.0	78.0
	1153	28.0	0.5	1.7	52.0	78.0
	1156	27.0	2.0	3.8	58.0	77.0
	1200	28.5	1.5	3.1	54.5	83.0
	1202	28.1	1.4	2.5	53.0	82.5
	1205	30.6	−0.7	0.5	51.5	81.0
	1207	31.7	−4.7	0.4	43.5	96.0
2	1223	28.1	1.4	1.6	59.0	54.0
	1226	29.3	−0.8	0.6	48.0	61.0
	1230	28.8	0.7	2.0	57.0	60.5
	1233	29.0	0.3	0.7	49.0	53.5
	1236	29.7	0.0	0.6	45.0	59.0
	1238	27.1	1.9	3.2	63.0	56.0
	1241	28.1	1.2	2.5	57.5	57.0
	1244	28.0	0.1	2.3	53.0	63.5
	1246	25.8	2.3	3.7	64.0	57.5
3	1302	29.5	0.2	3.4	49.5	89.0
	1304	28.8	2.7	6.3	54.0	74.0
	1306	27.7	2.2	4.3	57.0	72.0
	1309	28.1	2.5	3.6	54.0	76.5
	1313	27.5	2.2	4.4	56.0	77.0
	1315	30.3	0.9	2.8	47.0	86.0
	1323	28.1	4.3	7.2	73.0	76.0
	1328	30.3	0.9	3.4	50.0	72.0
	1335	28.1	4.6	8.9	60.0	70.0
	1337	30.5	−1.0	2.9	49.0	76.0
4	1347	28.8	2.3	0.7	52.0	65.0
	1350	29.5	1.0	0.4	64.0	88.0
	1353	26.9	2.1	2.4	58.0	61.5
	1355	27.0	1.1	1.8	57.0	67.0
	1400	27.7	0.4	1.1	53.0	67.0
	1403	29.3	−0.3	0.0	48.0	63.0
	1407	25.9	2.2	2.9	67.0	61.0
	1410	25.9	1.2	1.8	59.0	57.5
	1413	27.7	−0.2	0.4	48.0	68.0
	1415	26.4	0.0	0.6	50.5	66.0
5	1433	30.5	−2.4	−2.5	56.0	91.0
	1438	29.3	−0.8	−1.2	55.0	88.0
	1442	28.8	−0.8	−0.7	71.0	89.0
	1445	27.0	−0.1	1.0	73.0	89.0
	1448	27.0	0.1	1.0	67.0	79.0
	1450	29.3	−0.5	−1.3	53.0	70.0
6	1457	29.5	−1.5	−3.6	57.0	75.0
	1500	28.1	−1.6	−1.4	68.0	85.0
	1502	29.7	−1.7	−3.2	64.0	75.0
	1506	28.1	0.9	−0.1	76.0	77.5
	1510	30.3	−0.6	−1.8	71.0	79.0
	1513	30.3	−1.3	−1.8	64.0	77.5
	1517	29.5	0.0	−0.7	66.5	65.0
	1521	29.5	−1.0	0.0	65.0	68.0
	1524	31.8	−1.9	−2.3	55.0	76.0
	1527	32.2	−1.7	−2.3	53.0	73.0

Temperatures in Large Tents and Webs

During 1949, preliminary investigations of the effects of physical factors on the behaviour and activity of three species of tent caterpillars (*Malacosoma*) and of the spotless fall webworm, *Hyphantria textor* Harr., were begun. The equipment and techniques described in the present paper have been used in the general investigative routine. Although the main body of data being accumulated forms a separate subject which will be reported elsewhere, some of the routine measurements of temperatures taken in the silken tents are pertinent to the present work. Table 4 contains representative readings taken in and upon a tent formed by a colony of *M. pluviale* (Dyar) in early June.

TABLE 4.—TEMPERATURES, IN CENTIGRADE, UPON AND WITHIN A COLONIAL TENT OF THE WESTERN TENT CATERPILLAR, *Malacosoma pluviale* (DYAR)

Time: EST.	Air temperature	Tent surface temperature	Tent centre temperature
0800	12.6	13.6	17.5
0830	15.0	18.8	29.5
0900	15.2	15.7	23.6
0930	17.6	21.5	32.3
1000	18.8	23.5	35.6
1030	20.6	21.5	34.5
1100	24.2	24.7	35.5
1130	24.0	23.7	33.5
1200	23.7	24.7	36.0
1230	22.2	23.2	32.0
1300	22.0	26.0	32.7
1330	24.6	25.2	33.2
1400	23.3	23.2	34.0
1430	25.3	26.3	33.7
1500	25.6	30.5	39.7
1530	25.0	28.0	40.0
1600	22.5	24.3	33.5

The tabular values are representative of the differences which may be observed under a broken sky containing cirriform clouds and a few altocumulus clouds. The effects of these may be seen near the midday readings, especially.

Temperature readings taken within the webs of colonies of *Hyphantria* in early August show very similar values. Web temperatures in full sunlight range 8 to 13° above the air temperature measured at the same level, with values around 40° C. for an air temperature of 25-27° C. On clear nights, the webs are about 1° cooler than the air.

Studies of behaviour still in progress indicate that larval movements from tents or within them are governed to some extent by the fluctuations of physical factors within the tents.

DISCUSSION

It is clear from the foregoing descriptions that solar and nocturnal radiation exert decided effects upon the immediate habitats of insects which feed upon or within the parts of plants. Also, it is clear that the magnitude of the temperature differences observed is such that neglect of their existence may result in serious errors in attempts to relate laboratory and field results. Hitherto, entomologists appear to have recognized among such differences only those between air temperature and such values as subcortical temperatures of felled trees. To bark and cambial temperatures should be added those of buds, leaves, cones, flowers and, indeed, of the insects themselves. It has long been recognized that ordinary screen temperatures at times bear little relation to areas of entomological interest. However, the field situation is clearly very complex, and anyone wishing to obtain truly useful records must be prepared to work with something more than a mercury-in-glass thermometer. Blind acceptance of air temperature is a dangerous procedure, even when records are taken at a point of special interest. More attention should be paid to the behaviour of insects in their particular niches, and some effort must be made to analyse this behaviour in terms of changes in physical conditions, most of which (including evaporation) will be affected by radiation. Only when a good understanding of the behaviour mechanisms of a particular species has been reached can the relative importance of such factors as air temperature be correctly assessed.

It is worth re-emphasizing that, during initial tests to compare different types of instruments or different types of vegetation, comparable exposures are essential. This holds true not only in the vertical and horizontal planes at the periphery of any vegetation, but also in the space from the periphery to the interior of a tree. One finds a change in foliage climate a short distance inward from the periphery, particularly in a coniferous species. A brief inspection of the situation will show that the physical gradients inward toward a tree trunk are, in miniature, replicas of the more intense vertical gradients in the atmosphere from the forest floor to the canopy, the foliage in both situations acting to modify patterns of evaporation, convection and radiation, and, hence, of temperature. Some data on these points were accumulated in the present work, but the general situation in the forest is set forth so well in an earlier work (7) that repetition is needless.

SUMMARY

1. The observed differences between insect temperatures and the temperatures of the parts of plants on which they feed, on the one hand, and the temperature of the surrounding air, on the other, are sufficiently large to be given serious consideration in field studies.

2. The differences observed are largely a result of radiant heating by day and radiant cooling by night.

3. Under winter conditions, when the sun is at low elevations, heating is still sufficient to raise exposed coniferous foliage more than 2 Centigrade degrees above the surrounding air at latitude 46° 30′ North. By night, exposed foliage radiates down as much as 0.8° below the air temperature under a clear sky.

4. Snow-covered foliage remains above the air temperature on clear, calm nights, and below it during sunny days. This damping of daily fluctuations means that insects overwintering on branches beneath snow cover may experience about 8° less total change per day than insects on exposed branches.

5. Summer conditions give results somewhat similar to those observed during the winter, but values differ in degree. More intense incoming radiation by day raises vegetative parts as much as 8° above surrounding air, and more rapid rates of outgoing radiation at higher night temperature levels lower vegetation temperatures as much as 3° below the surroundings under a clear sky.

6. Wind does not modify temperatures elevated by radiant heating to a very marked degree, but it affects radiant cooling at night, particularly in winter when the decreased rate of cooling at generally low temperature levels is below that prevalent under summer conditions.

7. Winter cloud by day keeps exposed foliage slightly above air temperature, and by night re-emitted radiation from low cloud raises foliage temperatures fractionally above the air. Summer cloud by day permits elevation of foliage temperatures to 2° above the air. At night, an overcast seldom maintains foliage temperatures a significant amount above the surrounding air.

8. When isolated clouds obscure the sun, but not the rest of the sky, at any season, foliage radiates to levels below air temperature. Similarly, foliage exposed to the sky, but shielded from the sun, cools by radiation. If broken cloud is present, diffuse radiation keeps such foliage from cooling.

9. Spruce budworm larvae in their natural webs experience temperatures up to 8° above ambient conditions in midsummer.

10. Different parts of trees differ in their capacities to respond to radiant heating. Staminate flowers of coniferous trees commonly are at temperatures 5° to 8° above vegetative buds in sunlight. Since the buds, in turn, are above air temperature, this is an important difference for insects with habits like those of the spruce budworm.

11. The silken tents constructed by colonial species trap air which frequently ranges 8° to 13° above outside air in full sunlight.

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MOISTURE RELATIONSHIPS IN SOUTHERN ONTARIO

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In "The Climate of Southern Ontario", Putnam and Chapman (1) described the geographic distribution of the principal climatic elements of temperature and precipitation. The major part of the study was concerned with the temperature factor, although drought frequencies defined on an arbitrary basis were presented for the province, and Thornthwaite's original climatic classification (4) was used to compute the precipitation effectiveness indices for the three summer months. Thornthwaite's new classification (5) introduced a method of assessing the adequacy of precipitation by comparing it with the potential evapotranspiration or water need. In this way, times of water surplus and water deficiency can be determined and quantitatively expressed. An earlier article in this journal, "The climates of Canada according to the new Thornthwaite classification" (2) applied the method to the normals of the Canadian weather stations, and maps were presented of the new climatic elements of water need, water surplus and water deficiency. The same elements are presented on larger scale maps of southern Ontario in the first part of the present study.

Since normals do not express the all-important variability of the moisture factor in climate, the second part of the paper deals with the year-by-year variations in deficiency, surplus and climatic type at four representative stations in the southern part of the province.

PART I. AVERAGE MOISTURE RELATIONSHIPS

The general precipitation pattern in southern Ontario was presented in the earlier article by Putnam and Chapman (1). Precipitation ranges from 38 in. on the slopes east of Lake Huron and Georgian Bay and in the two eastern counties of Prescott and Glengarry, to less than 26 in. around Collingwood and in the extreme southwest of the province. Manitoulin Island and the Bruce Peninsula, Prince Edward County, the Niagara fruit belt and the Renfrew area annually receive less than 30 in. of precipitation

Potential Evapotranspiration

The concept of potential evapotranspiration or water need, introduced into climatological literature by Thornthwaite, has been discussed in previous publications (2) (3). Monthly or daily water need can be computed by means of a formula from the mean temperature modified by a length of day factor. The theoretical values of water need provided by the formula for southern Ontario have been verified by actual experiment (3). Equipment to measure the daily potential evapotranspiration from a grass-covered surface has been in operation in Toronto for three years and the results, given below, indicate the close correlation between measured water losses and those computed by means of the Thornthwaite formula.

TABLE 1.—POTENTIAL EVAPOTRANSPIRATION (CM.)

	1947		1948		1949	
	Measured	Computed	Measured	Computed	Measured	Computed
May	—	—	—	—	11.2	7.9
June	(6 days) 2.6	2.9	(18 days) 8.6	7.2	16.3	14.1
July	12.4	13.0	14.8	13.4	15.0	14.8
August	11.5	13.4	14.2	12.2	13.2	13.3
September	8.0	8.6	9.6	9.4	6.2	7.0
October	4.0	6.1	4.8	3.7	4.4	5.5
November	(14 days) 1.0	1.0	1.6	2.2	(22 days) 1.4	1.0
Total	39.5	45.0	53.6	48.1	67.7	63.6

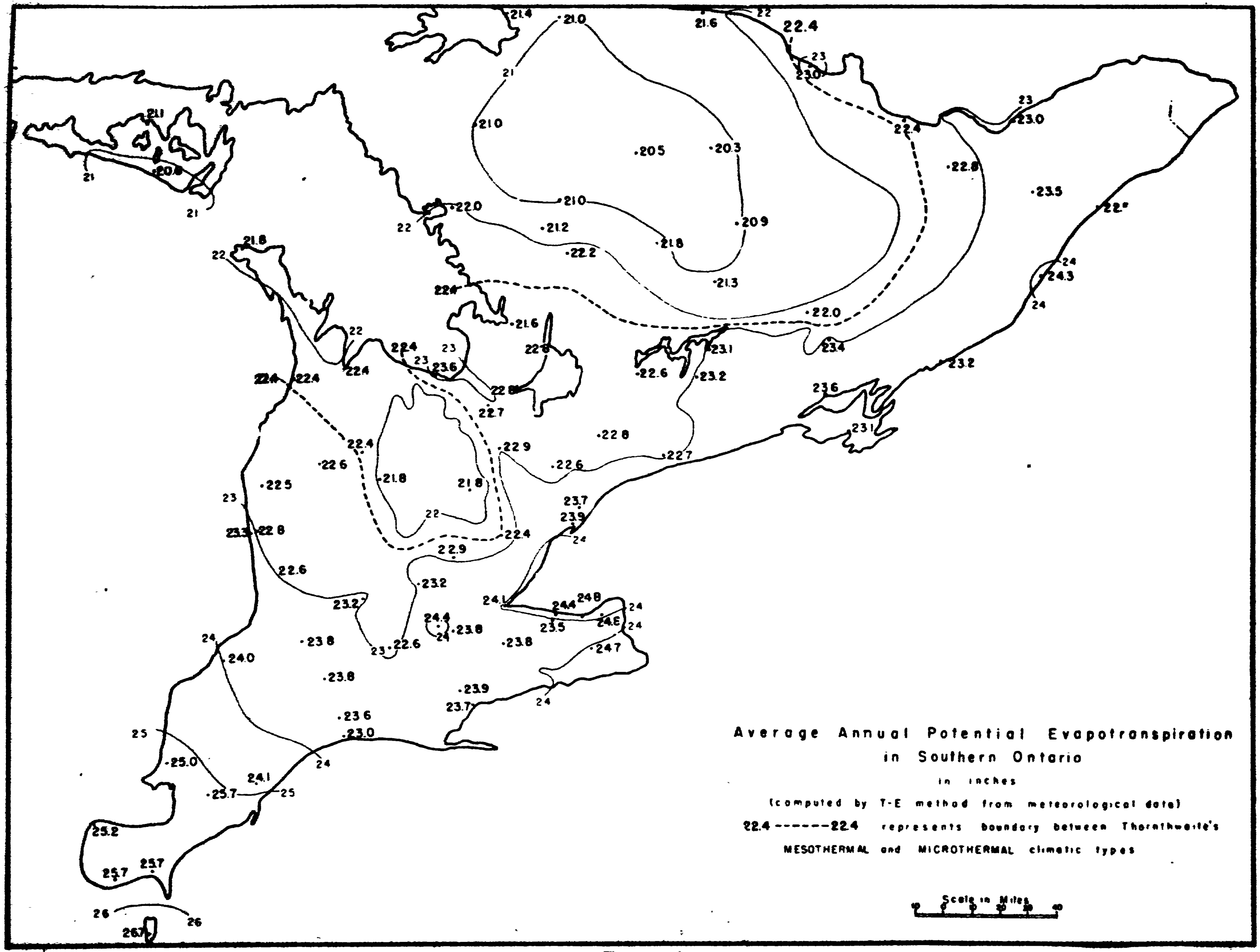
(The monthly totals represent the summation of the daily readings in each case.)

The annual potential evapotranspiration has been computed for the 83 weather stations in southern Ontario and its geographic distribution presented in Figure 1. These figures of water need express the amount of water annually needed for growth by the vegetation in each locality. They are also heat indices since they are derived from the mean monthly temperatures and take into consideration the varying lengths of day throughout the growing season. Potential evapotranspiration is thus a better expression of thermal efficiency than Thornthwaite's earlier, empirical T-E index (4), or an index of degree-days which expresses neither the increased growth at higher temperatures nor variation in day length.

Water needs of more than 25 in. per year are characteristic of Kent and Essex at the southwest tip of the province, and explain the great diversity of crops which can be grown there. With the exception of the interior valleys of southern British Columbia, water needs in that area are the highest in Canada. The average value of 26.7 in. for Pelee Island is the second highest in the Dominion. The western end of Lake Ontario and the Niagara fruit belt have similar high water needs of over 24 in., while the highlands east of Lake Huron and the Algonquin Park uplands have the lowest needs in southern Ontario, of less than 22 in. The heavy dashed line in Figure 1, representing a water need of 22.4 in., is an important boundary in the Thornthwaite classification, between the warmer mesothermal and cooler microthermal climatic types. The area which lies to the south of this line in Ontario, belonging to the mesothermal region, is the largest continuous area in Canada so climatically favoured.

Water Surplus and Deficiency

A figure of average annual water surplus can be obtained by applying the Thornthwaite formula to climatic data, and a further proof of the formula was supplied by the close correlation found to exist between computed water surplus and the measured run-off in the streams of southern Ontario (3). Water surplus is the result of precipitation in excess of need after the soil moisture has been replenished. It cannot be utilized by the vegetation since it runs off the surface or percolates through the soil to the ground water table. Figure 2 shows the distribution of average water



Average Annual Potential Evapotranspiration
in Southern Ontario
in inches
(computed by T-E method from meteorological data)
22.4 ------- 22.4 represents boundary between Thornthwaite's
MESOTHERMAL and MICROTHERMAL climatic types

FIGURE 1

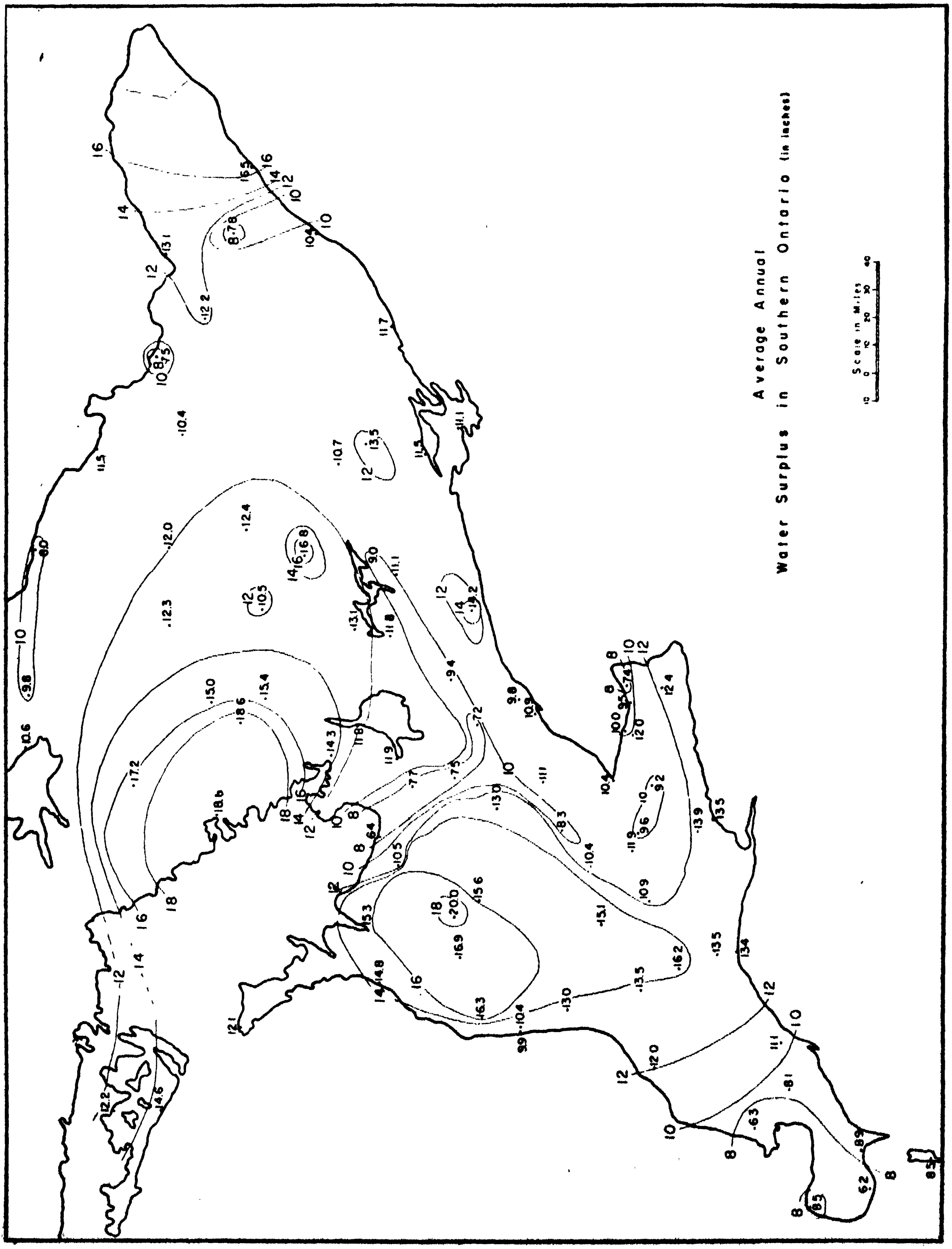

Average Annual
Water Surplus in Southern Ontario (in inches)
Scale in Miles
10 0 10 20 30 40

FIGURE 2

surplus in southern Ontario. Large surpluses of over 16 in. are characteristic of the area east of Georgian Bay, around Walkerton, Durham and Teeswater in Grey and Bruce counties, and in the eastern counties of Prescott and Glengarry. The Lake St. Clair-Harrow area and the St. Catharines region have small average surpluses of less than 8 in. A large average water surplus is not beneficial to the soil since it annually removes needed nutrients beyond the range of the plant roots. Large surpluses also make farming operations difficult and costly, by keeping the ground muddy and intractable in spring, delaying sowing operations and necessitating drainage. However, a surplus is agriculturally important in recharging ground water supplies and providing water for live stock and irrigation.

In most climatic classifications, southern Ontario has a humid climate, yet this fact does not exclude the possibility of drought. When the potential evapotranspiration exceeds the supply of moisture in the soil, moisture deficiency or drought occurs. The normal distribution of deficiency is shown in Figure 3. The west-central counties, the eastern tip of the province and the Algonquin Park uplands have average deficiencies of less than 1 in. Regions with deficiencies of more than 4 in. include Kent and Essex counties, the Prince Edward peninsula and the area below the escarpment in the Niagara peninsula. According to the Thornthwaite formula, 10 cm. or 4 in. is the amount of water available to the vegetation in the normal soil, and the above computed figures of deficiency were based on that assumption. However, there are some Ontario soils which could not hold 4 in.; and some which undoubtedly hold more than the 4 in. average. The shallow limestone soils of the Napanee plain, Prince Edward county, Manitoulin Island and the Bruce peninsula, belonging to the former group, thus probably suffer higher deficiencies than those shown in Figure 3; while deep black clay loams, such as those in Kent county, belong to the latter group, and actual deficiencies are doubtless lower than those shown in Figure 3.

Actual Evapotranspiration

The evapotranspiration or water loss that actually occurs in an average or a specific year can be computed from the meteorological statistics by using the Thornthwaite formula. Figure 4 shows the distribution of average actual evapotranspiration in southern Ontario. Actual evapotranspiration means potential evapotranspiration less water deficiency. Thus high water needs and low deficiencies characterize the areas where actual water losses are greatest, and, from the combined heat and moisture point of view, climatically best suited to agriculture. Highest average water losses occur in the Brantford, Woodstock, Stratford, and London region, and in the extreme east of the province, enclosed by the 22 in. isopleth (Figure 4). Harrow, with a high potential evapotranspiration of 26.7 in. has an actual water loss of only 19 in. in the average year. The lowest actual evapotranspiration in southern Ontario occurs on Manitoulin Island, where relatively low water need is combined with high deficiency. Since Figure 4 shows the geographic distribution of actual evapotranspiration, or the total water loss occurring during the average growing season, it best suits the purposes of correlation with yields of crops such as pasture grasses, whose growth is not affected by differences in planting dates.

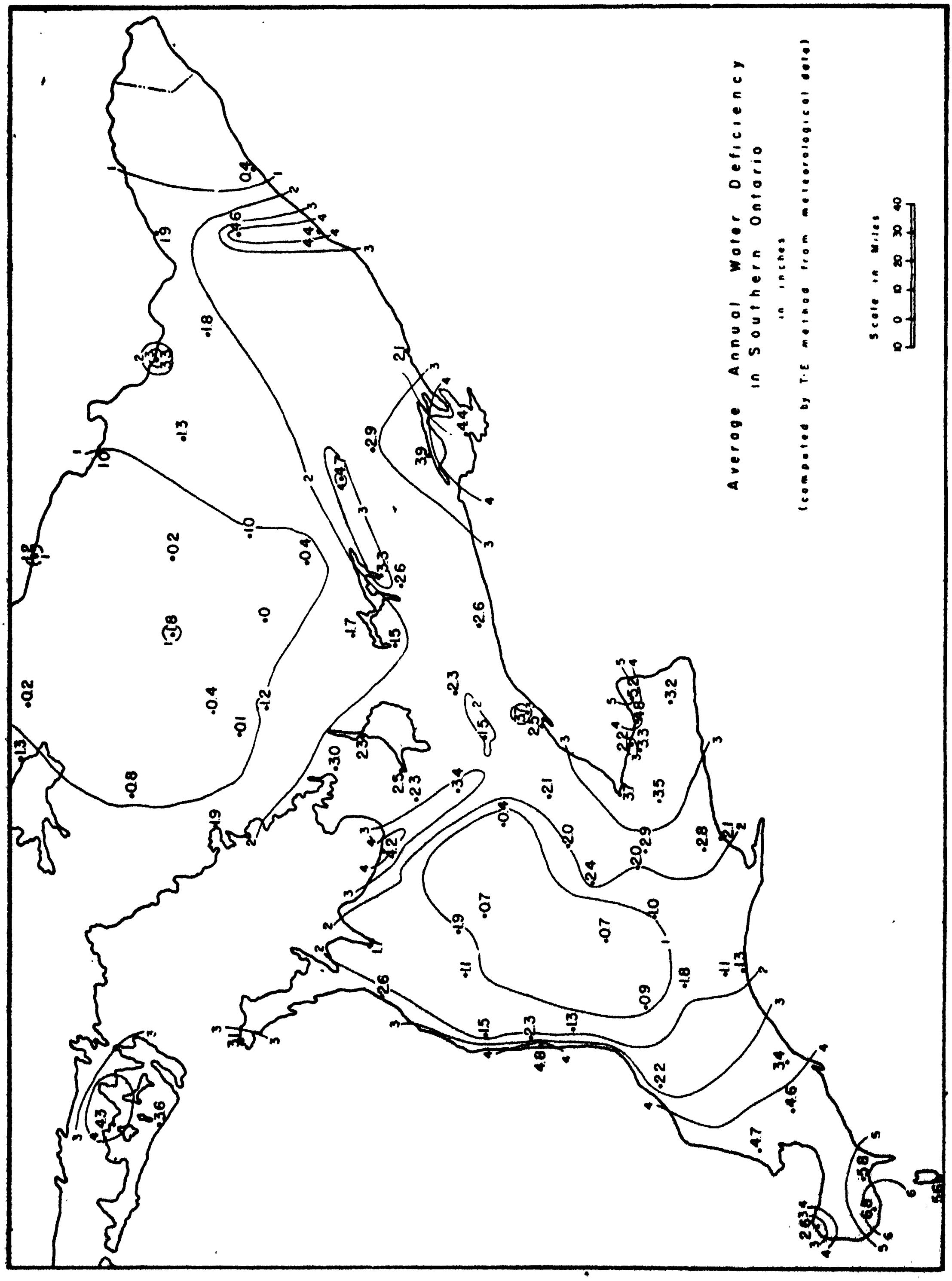

Average Annual Water Deficiency
in Southern Ontario
in inches
(computed by T-E method from meteorological data)
Scale in Miles
10 0 10 20 30 40

FIGURE 3

A Moisture Index

To satisfy the need for a rational climatic classification, Thornthwaite has devised a moisture index, relating water surplus and deficiency to need, on the following basis:

$$i = \frac{\text{surplus} - 0.6 \text{ deficiency}}{\text{need}}$$

For example, Toronto with a surplus of 10.9 in., a deficiency of 2.5 in., and a water need of 23.9 in., has an index of 39.3.

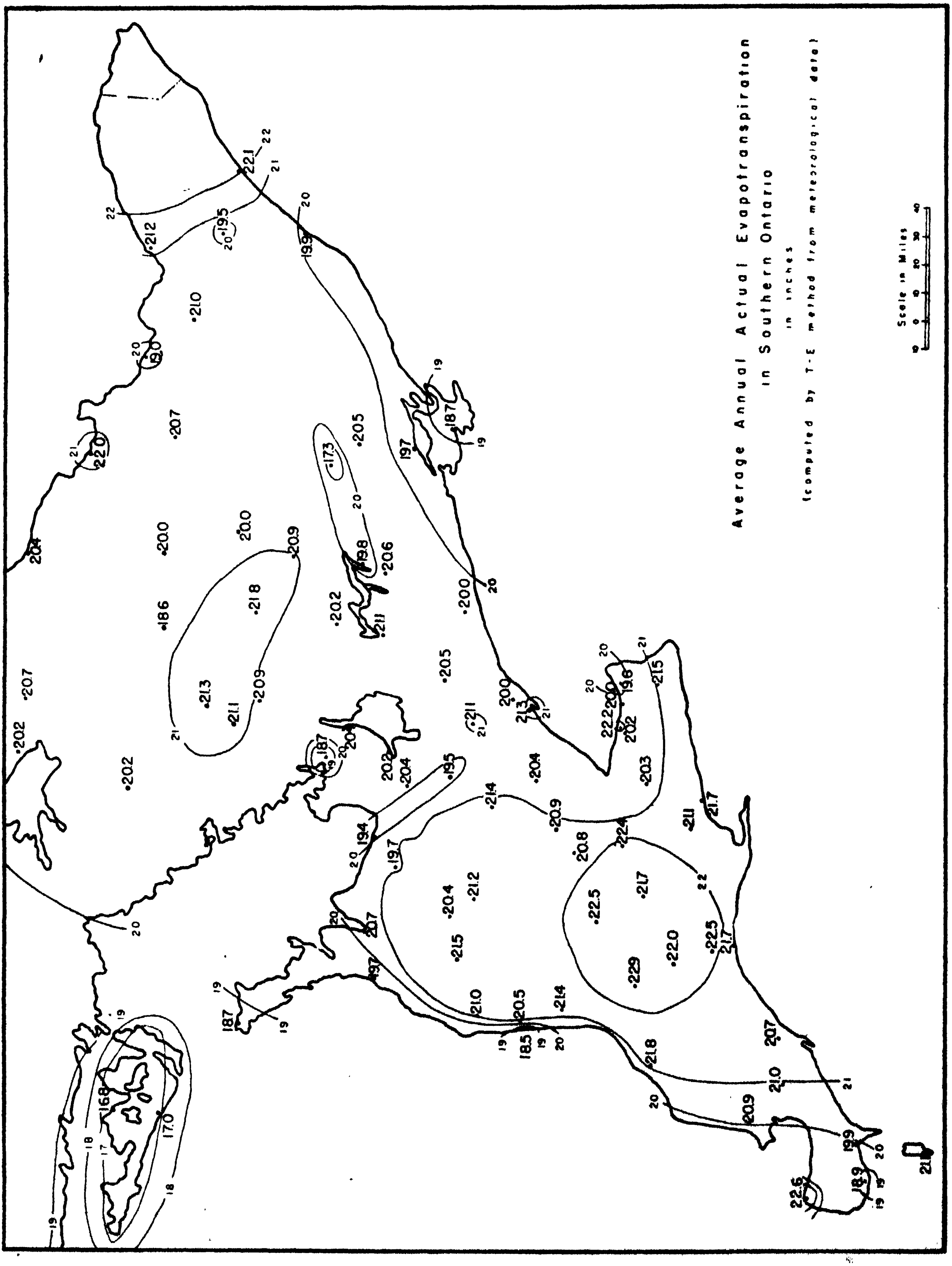
The Thornthwaite indices and corresponding climatic types appear below:

Symbol	Climatic type	Moisture index	Symbol	Climatic type	Moisture index
A	Perhumid	100 +	C_2	Moist subhumid	0 to 20
B_4	Humid	80 to 100	C_1	Dry subhumid	0 to −20
B_3	Humid	60 to 80	D	Semi-arid	−20 to −40
B_2	Humid	40 to 60	E	Arid	−40 to −60
B_1	Humid	20 to 40			

All nine moisture provinces in the Thornthwaite classification are found in Canada, although only five are represented in southern Ontario (see Figure 5). There are no areas of the perhumid A climates, found on the Pacific coast, nor do the three driest types C_1, D and E, characteristic of Western Canada and the interior valleys of British Columbia, appear in southern Ontario. All four humid climatic types, B_4, B_3, B_2, and B_1 appear in Figure 5 as well as the moist subhumid C_2 type. Beatrice is the most humid station, with an index of 87.5; Harrow the least humid, with an index of 8.4. This wide range in moisture conditions is illustrated by the fact that Charlottetown, P.E.I., has a moisture index of 85.4, while Sprague near Winnipeg, Manitoba (index 1.5) and Banff, Alberta (index 6.5) have moisture conditions similar to those at Harrow. Three areas are characterized by moist subhumid climates—Kent and Essex counties, the Collingwood area and the tip of the Niagara peninsula, while the most humid B_4 type is found in the areas with greatest surplus—east of Georgian Bay and in western Ontario around Durham. Graphs of five stations, representing each of the moisture provinces in southern Ontario, appear in Figure 6, and their comparative moisture data are given in Table 2.

TABLE 2

Moisture province	Station	Precipitation	Potential evapotranspiration	Actual evapotranspiration	Water surplus	Water deficiency	P. E. index
		(inches)	(inches)	(inches)	(inches)	(inches)	
Moist subhumid	Harrow	25.1	25.7	18.9	6.2	6.8	8.4
Humid B_1	Guelph	29.3	22.9	20.9	8.3	2.0	31.3
Humid B_2	Ottawa	34.2	23.0	21.1	13.1	1.9	52.0
Humid B_3	Walkerton	38.4	22.6	21.5	16.9	1.1	71.9
Humid B_4	Beatrice	39.6	21.2	21.1	18.6	0.1	87.5



Average Annual Actual Evapotranspiration in Southern Ontario in inches (computed by T-E method from meteorological data)

FIGURE 4

Moisture deficiencies steadily decrease and surpluses increase in Table 2 and Figure 6 from subhumid Harrow to most humid Beatrice. There is also a difference of approximately 4 in. in the annual water need between the least humid and the most humid station.

PART II. VARIABILITY OF MOISTURE

Method Used

While it is essential to know the average water relationships at any station, it is equally important to know the deviations from the normal conditions. Rainfall and water need vary from year to year as well as from place to place. To determine the frequencies of moisture deficiencies and surpluses of varying intensities, a study of daily moisture conditions was made at four selected stations for the 26-year period 1923 to 1948. Walkerton, Ottawa, Guelph and Harrow were chosen, since they represent the four major climatic zones; Harrow, Guelph and Ottawa have the additional advantage of being experiment stations. Correlations with actual crop data will thus be facilitated.

The Thornthwaite formula permits the computation of daily as well as monthly evapotranspiration, and by a simple bookkeeping procedure, treating precipitation as income and daily water need as outgo, the changes in soil moisture throughout the season can be determined. Figure 7 illustrates the soil moisture changes during 1948 in Harrow, assuming 10 cm. or 4 in. of moisture available in the soil for plant use. Moisture in excess of this is considered surplus and when the reserve is exhausted drought conditions are assumed.

It can be stated without exception that the soil in southern Ontario is everywhere saturated in early spring. Throughout January, February and March of 1948 at Harrow, precipitation greatly exceeded need, and since the soil moisture had been recharged the previous autumn, a moisture surplus of 14.2 cm. (5.6 in.) resulted (Figure 7). During April, the daily potential evapotranspiration totalled 5.6 cm. but 8.4 cm. of precipitation fell and at the end of the month the soil was still in a saturated condition. In early May, heavy rains resulted in more run-off, but after mid-May the soil began to dry out. By the end of May, the soil moisture level stood at 6.1 cm. During June, the moisture entering the soil from precipitation (11.2 cm.) was almost sufficient to supply the potential evapotranspiration needs (12.2 cm.) and the soil moisture fell slightly. In early July high water needs coupled with scant precipitation caused a rapid fall in soil moisture and by July 12 the moisture reserve was exhausted. The period of drought beginning July 12 lasted until August 9 with little relief. Water deficiency during this period totalled 7.9 cm. (3.1 in.). Again on August 26, the moisture level fell to 0 and the soil remained dry until September 5. A third period of deficiency began on September 15 and ended September 29. During October and November, current precipitation more than satisfied the needs of the vegetation and the reserve of moisture was again built up in the soil. The moisture deficiency for the growing season totalled 15.5 cm. (6.1 in.). This is below the average deficiency of 6.8 in. for Harrow and represents a year with better than average moisture conditions.

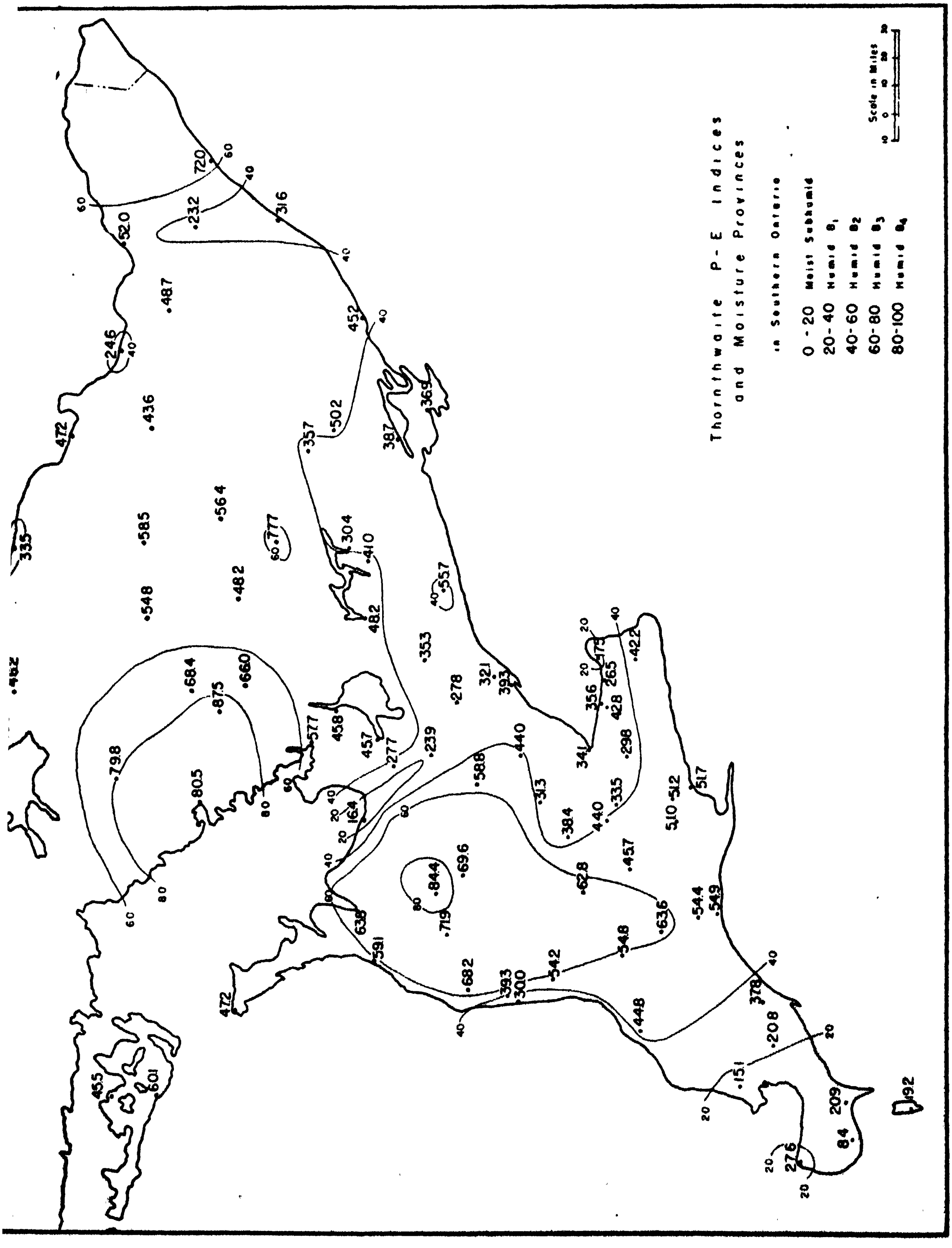

Thornthwaite P-E Indices
and Moisture Provinces
in Southern Ontario
0 - 20 Moist Subhumid
20 - 40 Humid B₁
40 - 60 Humid B₂
60 - 80 Humid B₃
80 - 100 Humid B₄
Scale in Miles
10 0 10 20 30
72.0
23.2
31.6
52.0
48.7
45.2
24.6
43.6
47.2
50.2
35.7
38.7
36.9
56.4
58.5
33.5
77.7
30.4
41.0
54.8
48.2
55.7
48.2
35.3
68.4
66.0
87.5
57.7
45.8
45.7
27.7
23.9
27.8
32.1
39.3
17.3
42.2
26.5
35.6
42.8
29.8
34.1
44.0
58.8
79.8
80.5
31.3
38.4
44.0
33.5
51.2
51.0
51.7
16.4
69.6
84.4
62.8
45.7
63.8
59.1
71.9
63.6
54.4
54.9
68.2
34.2
54.8
39.3
30.0
37.8
44.8
47.2
45.5
60.1
20.8
15.1
20.9
8.4
19.2
27.6
20
40
60
80

FIGURE 5

Variations in Precipitation and Water Need

The annual precipitation at each of the stations for the 26-year period is shown in Table 3, and a considerable variation is revealed. At Harrow the range was greatest, with a minimum in 1933 of 15.0 in. and a maximum in 1937 of 39.1 in. At Guelph, the precipitation ranged from 24.6 in. in 1930 to 41.9 in. in 1926. At Ottawa a maximum precipitation of 42.5 in. occurred in 1943 and a minimum of 24.3 in. in 1931. Walkerton's maximum for the period was 54.4 in. in 1928, the minimum 28.1 in. in 1944.

TABLE 3.—ANNUAL PRECIPITATION (IN INCHES), 1923-1948

	Harrow	Guelph	Ottawa	Walkerton
1923	27.07	35.84	36.44	—
1924	24.05	33.03	33.88	—
1925	26.71	33.70	37.22	36.82
1926	37.38	41.96	33.61	—
1927	—	37.05	40.28	36.78
1928	17.93	34.25	41.39	54.45
1929	26.15	34.24	34.85	43.64
1930	13.17	24.56	27.15	30.05
1931	17.56	31.28	24.30	33.47
1932	19.98	37.22	34.92	48.17
1933	14.96	27.71	34.34	34.97
1934	21.16	29.91	32.98	30.44
1935	28.40	27.63	29.49	32.05
1936	26.49	29.23	38.61	41.11
1937	39.06	36.11	37.04	38.52
1938	36.20	34.45	33.61	33.90
1939	29.54	25.70	37.47	39.65
1940	33.58	40.41	30.78	—
1941	27.89	28.39	29.70	33.51
1942	31.94	41.38	34.37	40.33
1943	28.68	30.96	42.50	38.75
1944	22.40	30.90	25.87	28.14
1945	31.97	40.84	40.59	38.62
1946	18.61	25.17	40.61	—
1947	32.79	32.44	40.06	—
1948	32.25	29.69	33.03	—
				1921—34.14
				1922—36.24*

* 1921 and 1922 used for Walkerton to compensate for gaps in study period.

While the variation in precipitation was great from year to year, the variation in potential evapotranspiration or water need was relatively small. The annual water need at Harrow, obtained as the sum of the daily water needs, varied from 30.5 in. in 1941 to 24.1 in. in 1924. At Guelph, 1941 again had the highest need of 26.1 in., 1924 the lowest of 21.8 in. The thermal range at Ottawa was similar to that at Guelph during the 26-year period, although the times of maximum and minimum were different. The highest potential evapotranspiration of 26.3 in. occurred here in 1923, the lowest of 21.9 in. in 1926. Walkerton with a shorter period of observation recorded a maximum of 26.5 in. in 1938 and a minimum of 22.9 in. in 1922.

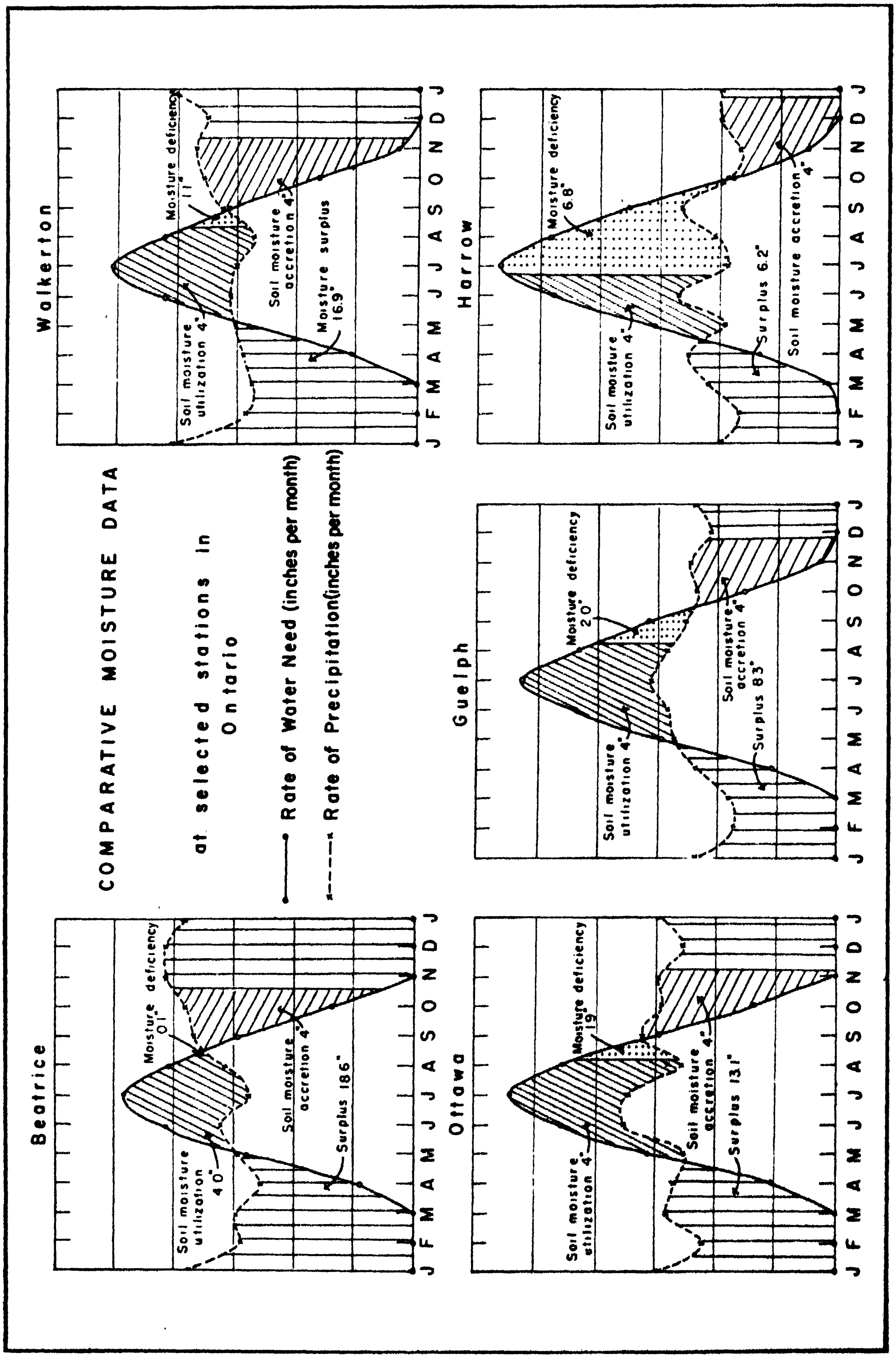

COMPARATIVE MOISTURE DATA
at selected stations in Ontario
Rate of Water Need (inches per month)
Rate of Precipitation (inches per month)
Walkerton
Soil moisture utilization 4"
Moisture deficiency 1.1"
Soil moisture accretion 4"
Moisture surplus 16.9"
Harrow
Soil moisture utilization 4"
Moisture deficiency 6.8"
Surplus 6.2"
Soil moisture accretion 4"
Guelph
Soil moisture utilization 4"
Moisture deficiency 2.0"
Soil moisture accretion 4"
Surplus 8.3"
Beatrice
Soil moisture utilization 4.0"
Moisture deficiency 0.1"
Soil moisture accretion 4"
Surplus 18.6"
Ottawa
Soil moisture utilization 4"
Moisture deficiency 1.9"
Soil moisture accretion 4"
Surplus 13.1"
J F M A M J J A S O N D J

FIGURE 6

Variations in Deficiency

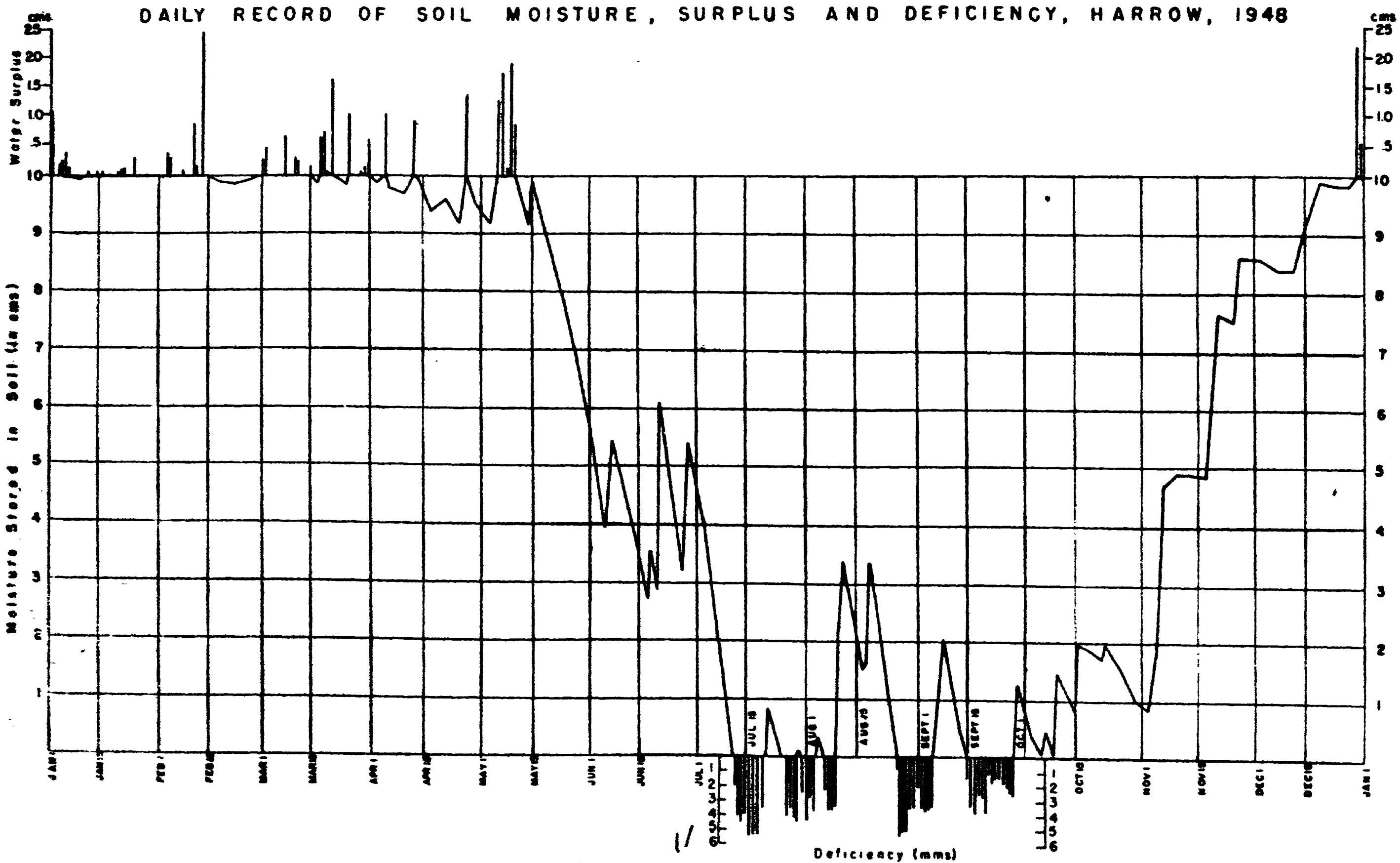
The Thornthwaite method as illustrated above for Harrow was used to determine the times and amounts of moisture deficiencies at each of the four stations during the 26-year period. The yearly deficiencies, obtained by totalling the daily values, appear in chronological order in Table 4, while Table 5 illustrates the frequency of occurrence of deficiencies of varying intensities, by 2 in. intervals.

TABLE 4.—MOISTURE DEFICIENCIES (IN INCHES), 1923-1948

	Harrow	Guelph	Ottawa	Walkerton
1923	7.80	0.70	4.04	5.64
1924	5.84	0.48	3.46	—
1925	7.61	1.87	3.96	4.58
1926	4.20	0.08	2.51	—
1927	9.35	2.15	1.89	3.43
1928	9.81	0	0	0
1929	9.34	5.96	3.12	5.31
1930	14.58	6.88	3.41	5.79
1931	12.90	4.06	9.20	3.84
1932	10.66	0.41	3.61	1.34
1933	16.21	8.35	5.78	8.64
1934	13.60	5.99	5.29	9.84
1935	5.40	4.72	3.23	4.00
1936	9.54	7.86	2.74	4.78
1937	1.53	0.79	2.82	4.45
1938	5.48	3.45	1.61	4.61
1939	7.62	9.22	1.34	1.13
1940	0.28	0.08	6.34	—
1941	6.57	5.87	8.48	8.27
1942	5.79	3.34	5.73	4.93
1943	7.02	3.02	0	1.27
1944	14.11	3.08	10.32	7.80
1945	6.29	3.15	1.61	2.74
1946	13.10	4.18	1.58	—
1947	7.30	5.68	3.10	—
1948	6.08	5.80	5.75	—
				1921—3.48
				1922—1.06

TABLE 5.—NUMBER OF YEARS WITH DEFICIENCIES OF VARYING INTENSITIES

Deficiency (inches)	Harrow	Guelph	Ottawa	Walkerton
0	0	1	2	1
0 - 1.9	2	7	5	4
2 - 3.9	0	6	10	4
4 - 5.9	5	8	5	9
6 - 7.9	8	2	1	1
8 - 9.9	4	2	2	3
10 - 11.9	1	0	1	0
12 - 13.9	3	0	0	0
14 - 15.9	2	0	0	0
16 - 17.9	1	0	0	0
Number of years' record	26	26	26	22



DAILY RECORD OF SOIL MOISTURE, SURPLUS AND DEFICIENCY, HARROW, 1948

FIGURE 7

A deficiency of moisture is always harmful to a crop, in reducing the yield and making the plant susceptible to disease. Walkerton, the most humid of the four stations, nevertheless had deficiencies ranging from 0 in 1928 to 9.8 in. in 1934. In 9 of the 22 years of record (see Table 5) total deficiencies amounted to less than 4 in., while only 4 years had deficiencies of more than 6 in. The average value was 4.4 in. for the study period, although the long-time average computed on a monthly basis was only 1.1 in. The fact that the long-term average deficiency at all four stations was lower than the 26-year deficiency based on daily values is an indication that the Thornthwaite formula used on a monthly basis underestimates moisture deficiency. The monthly basis cannot give an accurate value of deficiency since it fails to reflect the short droughts that occur within the month. This is the reason why it is necessary to make the daily computations.

Ottawa, representing the less humid B_2 climate, had an average deficiency of 3.9 in. during the 26-year period although on a monthly basis the average deficiency was 1.9 in. Deficiencies here ranged from 0 in 1928 and 1943 to 10.3 in. in 1944. Table 5 shows that the deficiency distribution at Ottawa has a well-defined peak in the 2 to 4 in. class. Of the four stations, Ottawa also had the greatest number of years (2). with no deficiency.

Guelph, with a less humid climate than Ottawa, had a similar deficiency distribution with a maximum of 9.2 in. in 1939. During the 26 years annual droughts averaged 3.7 in., while on a monthly basis the long-term average was 2.0 in. At Guelph, deficiencies of 0 to 2 in., 2 to 4 in. and 4 to 6 in. occurred with similar frequency and together accounted for 21 of the 26 years of record. For all 3 humid stations, Walkerton, Ottawa and Guelph, the frequency curve of deficiency is similar.

Harrow, representing the subhumid climatic type in southern Ontario, has deficiencies which may seem surprisingly high to those who consider Ontario a humid area. Here deficiencies varied from 0.3 in. in 1940, to as much as 16.2 in. in 1933. The average value for the 26-year period was 8.4 in. although on a monthly basis the average was 6.8 in. Table 5 shows that only 2 years of the 26 at Harrow had deficiencies of less than 4 in.; 7 years suffered droughts of more than 10 in.

The time when droughts occur as well as their intensity is important to agriculturists. A deficiency in June or early July, such as the one that occurred in 1949 throughout most of southern Ontario, causes the failure of early fruits and reduced yields of hay and grains; a deficiency later causes poor quality and reduced yield in vegetables, late fruits and tobacco. In any month drought causes the browning of pastures and the resulting problem of feed for livestock. Table 6 shows the number of occurrences of deficiencies of varying magnitudes by months at the four stations. At Harrow, Guelph and Ottawa, 26 years of records were used, but at Walkerton only 22 complete years were available.

It is noteworthy that at all stations deficiencies were experienced in five months of the year, and at Harrow and Guelph in six months. In no year was there water deficiency in May at any of the stations. However, June droughts occurred at Harrow in 11 years of the 26, and at Walkerton, Ottawa and Guelph in 3 or 4 years.

TABLE 6.—OCCURRENCE OF MONTHLY DEFICIENCIES OF VARYING MAGNITUDES DURING PERIOD 1923-1948

HARROW

Amount of deficiency	June	July	August	September	October	November
(inches)						
0	15	2	3	3	9	20
0 - 2	8	4	5	12	17	6
2 - 4	3	13	15	11	0	0
4 - 6	0	7	3	0	0	0

GUELPH

0	23	11	5	6	17	25
0 - 2	3	9	12	16	9	1
2 - 4	0	4	9	4	0	0
4 - 6	0	2	0	0	0	0

OTTAWA

0	22	9	5	7	21	26
0 - 2	4	10	10	18	5	0
2 - 4	0	7	9	1	0	0
4 - 6	0	0	2	0	0	0

WALKERTON

	Years of record					
	(23)	(23)	(23)	(22)	(22)	(22)
0	19	8	4	6	15	22
0 - 2	3	7	5	14	7	0
2 - 4	1	7	13	2	0	0
4 - 6	0	1	1	0	0	0

July is the month when highest deficiencies may be expected in the Harrow and Guelph area. At Harrow, only 2 years showed no July deficiency, but 7 had deficiencies of more than 4 in. The magnitude of this deficiency is apparent when it is realized that the average potential evapotranspiration in July is only 5.5 in. Although 11 years at Guelph showed no July deficiency, 2 years had deficiencies of more than 4 in. Ottawa was the only station of the four where a July deficiency of 4 in. was not recorded although only about one-third of the July records showed no deficiency. Similarly at Walkerton, about one-third of the years had no July deficiency. In August, Ottawa and Walkerton can expect their highest deficiencies and at all four stations it seems to be the month with the least likelihood of escape from drought. In only 5 years during the period of observation was there no deficiency in August at Guelph and Ottawa; in only 3 years at Harrow. In the majority of years, southern Ontario can expect drought in September, although deficiencies are smaller in magnitude since water needs are smaller. At Ottawa, Walkerton and Guelph there were 6 or 7 years without moisture deficiency during September; at Harrow only 3. October is usually considered a humid month in southern Ontario with precipitation adequate for the needs of the vegetation; yet all four stations recorded deficiencies during October—in 5 years at Ottawa and in 17 years at Harrow. By November the water needs are greatly reduced and at no time during the study period did droughts occur at Walkerton or Ottawa. Slight deficiencies were experienced in the Guelph and Harrow area during November.

The small chance of escaping deficiency in any year in southern Ontario lends support to the theory that crop yields in the so-called humid East are reduced every year by invisible drought to as little as 35 per cent of the potential (6). In 1949, lack of moisture was the cause of a complete failure of the strawberry crop in most of southern Ontario. The obvious means of combating deficiency is by irrigation, and in this respect the Thornthwaite method provides a most worth-while tool. Irrigators can use the formula to determine the daily water loss from a crop, and thus the daily changes in soil moisture (as illustrated in Figure 7 for Harrow). Before the moisture reserves reach a dangerously low level, additional water to a known depth can be supplied. By following this system two

TABLE 7.—WATER SURPLUSES (IN INCHES), 1923-1948

	Harrow	Guelph	Ottawa	Walkerton
1923	9.46	12.42	14.22	—
1924	7.93	11.71	13.72	—
1925	6.47	12.89	16.94	18.26
1926	17.35	20.90	14.23	—
1927	—	16.24	18.11	16.55
1928	3.11	11.48	17.71	30.65
1929	8.74	17.96	15.46	25.50
1930	3.80	7.75	6.61	10.35
1931	1.39	9.32	7.63	11.18
1932	0.97	14.13	14.91	26.15
1933	3.35	11.06	16.16	17.71
1934	5.51	11.44	14.85	14.93
1935	6.82	8.49	9.93	12.38
1936	8.50	12.79	18.16	21.38
1937	14.42	14.30	15.56	18.39
1938	12.84	10.93	10.85	12.02
1939	12.06	11.44	16.36	16.85
1940	5.58	16.84	14.50	—
1941	4.29	8.20	13.17	15.59
1942	9.17	20.80	14.83	20.19
1943	10.24	11.09	19.94	16.61
1944	5.91	9.95	10.89	9.67
1945	8.99	19.78	17.78	16.43
1946	3.90	5.46	17.87	—
1047	10.53	13.06	19.26	—
1948	10.37	10.08	13.39	—
				1921—12.85
				1922—14.37

TABLE 8.—NUMBER OF YEARS WITH SURPLUSES OF VARYING AMOUNTS

Surplus	Harrow	Guelph	Ottawa	Walkerton
(inches)				
0 - 4.9	7	0	0	0
5 - 9.9	11	6	3	1
10 - 14.9	6	14	11	7
15 - 19.9	1	4	12	8
20 - 24.9	0	2	0	2
25 - 29.9	0	0	0	2
30 - 34.9	0	0	0	1
Number of years' record	25	26	26	21

faults common among irrigators can be corrected—that of waiting until the plants show signs of deficiency before irrigating and that of applying too little or too much water. An irrigation system using the Thornthwaite method and applying water when the soil moisture reserves were depleted 1 in. has been used on red pine seedlings during the 1948 and 1949 seasons at the Provincial Forestry Station at Orono with substantial and gratifying increase in growth. Such a system could be applied equally well to agricultural crops such as corn, spinach or pasture grasses.

Variations in Moisture Surplus

During the cooler half-year in southern Ontario, precipitation greatly exceeds water need, and after the soil moisture has been recharged, a moisture surplus occurs. The geographic distribution of average annual surplus was shown in Figure 2. Much of the surplus water in southern Ontario occurs as snow and does not run off or percolate to the ground water table until spring. Surpluses are shown by calendar years at the four selected stations in Table 7. Table 8 shows the frequency of occurrence of surpluses of varying amounts.

There are as wide variations in water surplus throughout the 26-year period as there were in moisture deficiency. At Harrow the surplus may be as little as 1.0 in. (1932) or as great as 17.4 in. (1926)—a variation of more than 16 in. In 7 of the 26 years of record at Harrow the moisture surplus amounted to less than 5 in. and in only one year was it more than 15 in. These surpluses are considerably lower than for the other three stations. At Guelph, the variation in surplus was from 5.5 in. in 1946 to 20.9 in 1926—a range of 15 in. A surplus of less than 5 in. never occurred here, and 6 years recorded surpluses of more than 15 in. Ottawa had the smallest variation in moisture surplus of the four stations—from 6.6 in. in 1930 to 19.3 in. in 1947. Twenty-three of the 26 years of record had surpluses between 10 to 20 in. Although both average precipitation and average surplus are highest in Walkerton, the variation in surplus is also greatest—from 9.7 in. in 1944 to 30.6 in. in 1928. In Walkerton, as in Ottawa, the most frequent occurrences were in the 10 to 20 in. classes, but unlike Ottawa, 5 years recorded surpluses of more than 20 in.

While the chief importance of water surplus in agriculture lies in the leaching and intractability of the soil, a knowledge of the magnitude of the water surplus and its variation from year to year is most important in understanding the hydrological cycle. The present 26-year study illustrates the variability of run-off that can be expected at four stations representative of the four major moisture regions in southern Ontario. In addition, the method used here to obtain yearly figures of water surplus from the meteorological statistics could be useful to hydrologists in estimating run-off and its variation in areas where no run-off measurements are made or where only short records are available. Worth-while correlations could also be made with changes in ground water levels.

Variability of Moisture Index and Climatic Type

Thornthwaite's moisture index was devised to express the relationship between surplus and deficiency and water need; and the geographic distribution of indices and resulting moisture provinces were seen in Figure 5. A humid B climatic region, however, may have a perhumid A climate in one year and a subhumid C_2 climate in another; similarly, a subhumid C area may be semi-arid one year and humid another. The yearly moisture indices for the four stations with the corresponding Thornthwaite climatic types are seen in Table 9, while Table 10 shows the number of occurrences of each climatic type during the 26-year period. Six climatic types occurred at Harrow and Walkerton, and five at Guelph and Ottawa.

Harrow belongs to the C_2 climatic type; yet of the 25 complete years of record only 9 had typical C_2 moisture conditions. Drier C_1 conditions were characteristic of 5 years and a semi-arid D climate typified 1931, 1932 and 1933. Moister-than-average B_1 conditions were typical of 6 years but B_2 and B_3 climates occurred in only 2 of the 25 years.

TABLE 9.—MOISTURE INDICES AND CLIMATIC TYPES, 1923-1948

	Harrow	Guelph	Ottawa	Walkerton
1923	18.7 C_2	47.0 B_2	44.8 B_2	(1921) 42.2 B_2
1924	18.4 C_2	45.1 B_2	49.2 B_2	(1922) 60.0 B_3
1925	7.4 C_2	46.3 B_2	57.3 B_2	67.2 B_3
1926	61.0 B_3	99.0 B_4	60.2 B_3	—
1927	—	65.2 B_3	70.6 B_3	61.2 B_3
1928	−10.8 C_1	50.4 B_2	74.6 B_3	129.0 A
1929	16.8 C_2	64.5 B_3	60.4 B_3	95.0 B_4
1930	−18.3 C_1	14.9 C_2	19.1 C_2	26.9 B_1
1931	−22.4 D	27.0 B_1	8.2 C_2	34.0 B_1
1932	−21.4 D	59.0 B_2	53.9 B_2	108.5 A
1933	−21.8 D	24.2 B_1	53.0 B_2	48.5 B_2
1934	− 9.0 C_1	31.9 B_1	49.6 B_2	36.0 B_1
1935	13.9 C_2	23.7 B_1	35.1 B_1	42.1 B_2
1936	10.1 C_2	33.3 B_1	71.4 B_3	75.5 B_3
1937	51.6 B_2	57.8 B_2	57.0 B_2	64.0 B_3
1938	33.1 B_1	34.5 B_1	40.5 B_2	34.9 B_1
1939	27.1 B_1	23.7 B_1	68.0 B_3	67.5 B_3
1940	21.1 B_1	76.0 B_3	47.2 B_2	—
1941	1.4 C_2	18.0 C_2	32.3 B_1	40.6 B_2
1942	20.5 B_1	78.8 B_3	44.6 B_2	68.6 B_3
1943	22.2 B_1	40.5 B_2	88.5 B_4	67.5 B_3
1944	− 8.5 C_1	33.6 B_1	18.5 C_2	18.8 C_2
1945	18.4 C_2	73.9 B_3	68.8 B_3	59.5 B_2
1946	−13.5 C_1	12.1 C_2	69.8 B_3	—
1947	21.9 B_1	38.8 B_1	72.7 B_3	—
1948	16.9 C_2	26.2 B_1	39.1 B_1	—

TABLE 10.—OCCURRENCE OF THORNTHWAITE CLIMATIC TYPES, 1923-1948

Climatic type	Harrow	Guelph	Ottawa	Walkerton
Perhumid A	0	0	0	2
Humid B_4	0	1	1	1
Humid B_3	1	5	9	8
Humid B_2	1	7	10	5
Humid B_1	6	10	3	4
Moist subhumid C_2	9	3	3	1
Dry subhumid C_1	5	0	0	0
Semi-arid D	3	0	0	0
Arid E	0	0	0	0
Number of years' record	25	26	26	21

Guelph is typically a B_1 climate and 10 years of the 26 had B_1 moisture conditions. A drier C_2 climate was typical of only 3 years while moister B_2 and B_3 conditions occurred in 12 years. The year 1926 had an exceptionally moist B_4 climate.

Ottawa, chosen to represent the B_2 climatic type, had a typical B_2 climate in 10 years of the 26. Three years had drier B_1 conditions and 3 years subhumid C_2 conditions. In 10 years moister-than-average climatic conditions prevailed; 9 were typically B_3 while one (1943) had a B_4 climate.

Walkerton belongs to the B_3 climatic type, and of the 21 complete years of record here, 8 had typical B_3 moisture conditions. Nine years were characterized by less humid B_1 and B_2 conditions and one (1944) by subhumid C_2 conditions. A moister-than-average B_4 climate was typical of 1929 and a perhumid A climate of 1928 and 1932. Walkerton was the only station where a perhumid A climate occurred during the years under observation and Harrow the only station where semi-arid D climates were experienced.

SUMMARY

The present study with the aid of maps and tables has presented the normals and the yearly variations in the moisture relationships in southern Ontario's climate. The geographic distributions of average water need, deficiency, surplus and actual water loss as computed by the Thornthwaite method were shown and the province divided into five major moisture regions. The greater part of southern Ontario belongs to the humid climatic province, but to the less humid B_1 and B_2 rather than the more humid B_3 and B_4 types. A considerable area in the southwest of the province is climatically subhumid.

Four stations—Harrow, Guelph, Ottawa and Walkerton—were chosen to represent the four main moisture provinces, and their daily changes in soil moisture computed for the years 1923 to 1948. Variations in precipitation and water need during the period were shown, and the subsequent variations in water deficiency and water surplus. Deficiency was treated in detail since it is the climatic factor most important in reducing crop yields in the province. At each station only one or two years during the

26-year period had no deficiency, while at Harrow deficiencies ranged as high as 16 in. per year. The frequency of occurrence of annual deficiencies of varying intensities was analysed at the four stations. In addition, the chances of deficiency during every month of the growing season were studied to indicate the times of greatest and least drought frequency.

The daily changes in soil moisture during 1948 at Harrow were plotted graphically to illustrate how the Thornthwaite method can be used in working out an irrigation system to supply supplementary water to a crop before it suffers from deficiency and yield is reduced.

Yearly variations in moisture surplus at the four stations were similarly presented, and when analysed by the hydrologists could help to explain seasonal variability in run-off and changes in ground water levels.

Finally the Thornthwaite moisture indices, relating water deficiency and surplus to need in each year were computed to indicate the number of years when average, wetter-than-average, and drier-than-average moisture conditions could be expected in the four localities.

Since three of the four stations studied are government experiment stations, it is hoped that the detailed moisture data provided here will be used in correlating climate with the growth, yield and quality of crops and with the occurrence of plant diseases and insect infestations.

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RECENT FIELD TESTS ON THE EFFECT OF SOIL FUMIGANTS UPON BROWN ROOTROT OF TOBACCO IN ONTARIO

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The testing of soil fumigants, for the control of brown rootrot of tobacco has recently been reported from most of the more northern tobacco-growing states of the United States. In Connecticut, Anderson (1) and, in North Carolina, Nusbaum and Todd (3) report the successful use of ethylene dibromide and a mixture of dichloropropylene and dichloropropane. In Kentucky, Valleau (4) reports non-beneficial results with one fumigant. Kincaid (4) reports that soil fumigation does not control meadow nematodes as well as it does root knot nematodes. The effect on quality has been variously reported by different workers.

EXPERIMENTAL

For several years past the effect of various soil fumigants upon the growth of burley tobacco in typical "brown rootrot" soil has been investigated at Harrow, Ontario. Exploratory tests conducted for two years prior to 1949 showed consistent but variable increase (up to 300 per cent) in growth of tobacco on brown rootrot soil to which either dichloropropylene-dichloropropane mixture or ethylene dibromide was applied broadcast at either 20 or 30 gal. per acre.

During the current year it became possible to conduct soil fumigation tests under highly favourable conditions. A plot (235′ × 150′) of Fox sandy loam soil carefully selected because of its record for occurrence of brown rootrot was used for the experiment. A heavy crop of corn which in Ontario has always proved to be the crop most conducive to severe brown rootrot in the subequent tobacco crop, was produced on this plot in 1948 and the entire growth, including stalks, was ploughed under in the spring of 1949. Six chemicals were used, namely, methyl bromide (xylene base), methyl bromide (carbon tetrachloride base), chloropicrin, ethylene dibromide, and dichloropropylene-dichloropropane mixture. All chemicals were applied with a common type (Mack) hand injector in the tobacco row 10 days prior to planting, the injections being made at a depth of 4–5 inches at 8-inch intervals along rows 40 inches apart. The soil had been ploughed, disked, and cultipacked previous to treatment and was moderately moist at the time of application of the chemicals. No seal was used. No rainfall occurred between May 30, the date of application, and June 9, when the tobacco was planted by machine. The plot was divided into four separate ranges and single alternate rows of the varieties Harrow Velvet and Green Briar were planted in each range. Harrow Velvet is completely susceptible to brown rootrot (2) while Green Briar is the most resistant of the burley varieties commonly grown in Ontario. In most cases there were five randomized replicates of each treatment. A single test unit comprised 18 plants. Treatments are listed in Table 1 and results are summarized in Tables 2 and 3.

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[2] Senior Pathologist-in-Charge.
[3] Assistant Pathologist.

TABLE 1.—LIST OF FUMIGANTS APPLIED TO FIELD SOIL INFESTED WITH BROWN ROOTROT OF TOBACCO AND AMOUNTS USED

Treatment designation	Fumigant	Amount (cc.) per 8-inch centre
MB_1	Methyl bromide (15%) (xylene base)	6*
MB_2	Methyl bromide (15%) (xylene base)	10
MB_3 Dowfume G	Methyl bromide (10%) (Carbon tetrachloride base)	10
Dow W40	Ethylene dibromide	6
Cp	Chloropicrin	6
Dow N	Dichloropropylene-dichloropropane	6
P_1	Parathion	6
P_2	Parathion	10

* 6 cc. per 8-inch centre equivalent to 30 gal. per acre.

TABLE 2.—RELATIVE ROOT INJURY ON BURLEY TOBACCO IN BROWN ROOTROT FIELD PLOTS TO WHICH DIFFERENT SOIL FUMIGANTS WERE APPLIED BY ROW INJECTION METHOD*

Treatment

MB**	P_1	P_2	MB_1	MB_2	Dow W40	Dow N	Cp	Checks	
								Green Briar	Harrow Velvet
				Percentage root injury					
20	100	55	75	10	40	5	5	20	70
100	30	90	10	40	5	5	5	15	100
80	95	100	5		15	10	5	20	90
	60	40	40		10	10	5	50	100
	40	30	30		30	15		50	100
				Average root injury					
66	65	63	32	25	20	9	5	31	92

F = 11, L.S.D. at 5% = 2.7.

* Based on macroscopic examination of roots of 6 random plant samples from each of 5 replications. Higher figures represent greater root injury.

** For key to treatments, see Table 1.

An examination of averages in Table 2 indicates marked differences in root injury among plots of the susceptible variety Harrow Velvet treated with the various soil fumigants. Average root injury was less on all fumigated than on non-fumigated plots of the susceptible variety, regardless of the chemical used. As will be noted, root injury varied from as little as 5 and 9 per cent in the chloropicrin- and Dow N-treated plots, respectively, up to 65 and 66 per cent in the plots treated with Parathion and MB_3, respectively. In non-treated soil, root injury averaged 31 per cent in the resistant Green Briar variety and 92 per cent in the susceptible Harrow Velvet. Thus, the average root injury on the resistant variety in non-treated but infested soil occupied a position approximately midway between the greatest and the least average injury among the fumigated plots of the susceptible variety.

TABLE 3.—GREEN WEIGHT YIELDS OF BURLEY TOBACCO IN BROWN ROOTROT FIELD PLOTS TO WHICH DIFFERENT SOIL FUMIGANTS WERE APPLIED BY ROW INJECTION METHOD*

Treatment

Dow N	Chloropicrin	Dow W40	MB_1	MB_2	P_1	MB_3	P_2	Checks	
								Green Briar	Harrow Velvet
					lb.				
16	20	12	3	3	1	7	2	20 plots	14 plots
23	23	13	15	6	4	2	1		
22	7	7	13		1	1	2		
11	13	20	16		8		4		
20		18	12		7		4		
				Total weight (lb.)					
92	63	70	59	9	21	10	13	360	280
				Av. lb. per plot					
18.4	15.6	14.0	11.8	4.5	4.2	3.3	2.6	18.0	2.0

F =9.4, L.S.D. at 5% =2.7.

* Six plants were weighed in each plot at "topping" stage.

From Table 3, it is evident that a wide range of green-weight yields prevailed among the fumigated plots of the susceptible variety. These range from 2.6 lb. in the Parathion-treated plots to 18.4 lb. in the Dow N-treated plots. Among the non-treated plots, yields averaged 18.0 lb. in the Green Briar plots (Figure 1, B; Figure 2, B; and Figure 3, A, C, and E) and only 2 lb. in the Harrow Velvet plots (Figure 1, C; Figure 2, C; and Figure 3, B and D). Thus, as far as the susceptible variety is concerned, the least growth of all occurred on the non-treated plots. On the other hand, the green weight yield of the susceptible variety in plots treated with Dow N (Figure 2, A) was slightly greater than that of the resistant variety in non-treated soil. Thus, the application of Dow N to brown rootrot soil resulted in an increase of 920 per cent for the susceptible variety.

Table 4 shows that, in treated rows of Green Briar yields of 11 and 18 lb. in the Dow N and Dow W40 plots, respectively, were obtained. These yields, it will be noted, are no higher than those in untreated rows of the same variety (see Table 3). On the other hand, the yield of Harrow Velvet—1 and 13 lb. in the Dow N and Dow W40 plots, respectively, in fumigated soil—represents an increase of 11 to 13 times that of adjacent checks of the same variety.

Statistical analyses of results in Tables 2 and 3, except for MB_2 and MB_3, where an insufficient number of plants were available for inclusion, showed significant differences both in root injury and in green weights between all fumigation treatments. A correlation was also indicated between root injury and green weights though the coefficient of correlation (−.41) was not so high as might be expected, probably because only a single set of root observations could be made.

It should also be mentioned that from the time late in June, when plot differences first became apparent, brown rootrot was obviously severe on Harrow Velvet throughout the entire field experiment where soil fumigants

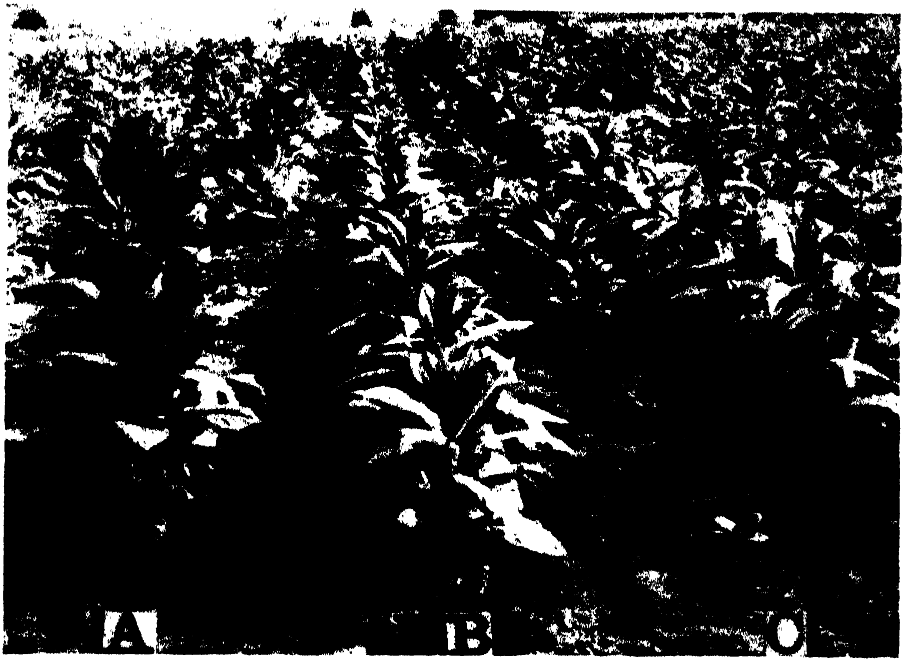

FIGURE 1. Brown rootrot soil fumigation plots. A—Harrow Velvet (chloropicrin-treated). Some plants had been harvested from this row before photograph was taken. B—Green Briar (no treatment). C—Harrow Velvet (no treatment). Note severe stunting.

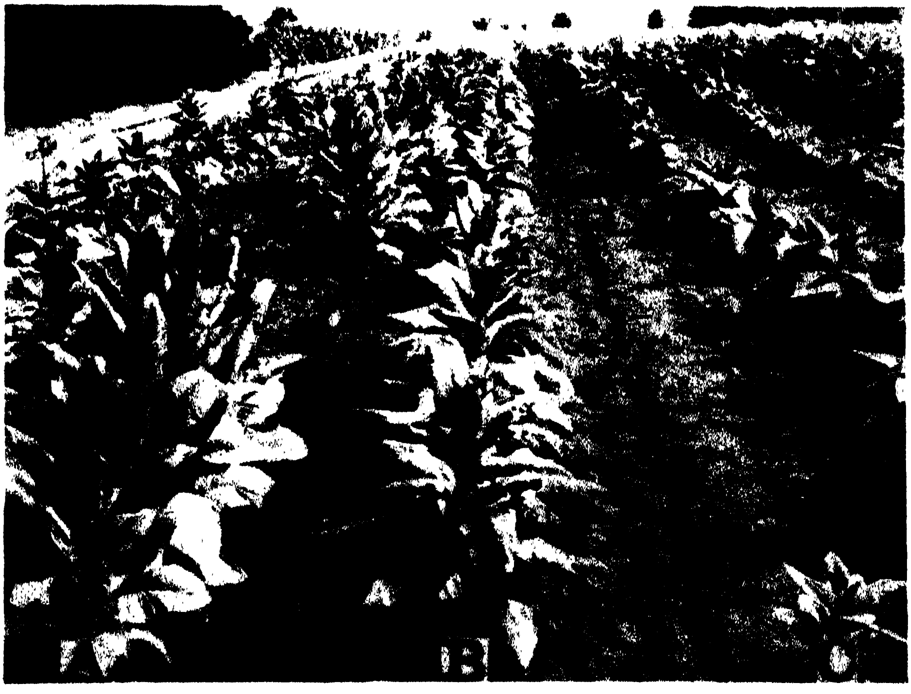

FIGURE 2. Brown rootrot soil fumigation plots. A—Harrow Velvet (Dow N-treated). Some plants had been harvested from this row before photograph was taken. B—Green Briar (no treatment). C—Harrow Velvet (no treatment). Note severe stunting.

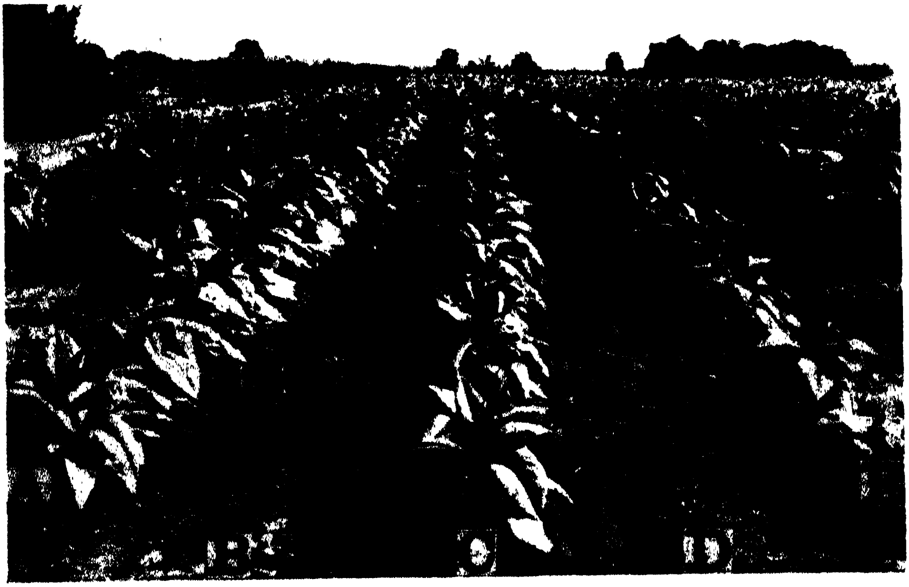

FIGURE 3. Brown rootrot soil fumigation plots. A—Green Briar (no treatment). B—Harrow Velvet (no treatment). C—Green Briar (no treatment). D—Harrow Velvet (no treatment). E—Green Briar (no treatment). N.B.—Green Briar resistant. Harrow Velvet susceptible.

TABLE 4.—GREEN WEIGHT YIELDS OF SINGLE PLOTS OF BURLEY TOBACCO IN BROWN ROOTROT FIELD SOIL WHERE FUMIGANTS WERE APPLIED BY BROADCAST METHOD*

Treatment	Variety		Check
	Harrow Velvet	Green Briar	Harrow Velvet
		lb.	
Dow N	11	11	1
Dow W40	13	18	1

* 4 cc. per injection; spacing 8 × 10 inches.

were not used. Many rows of this variety remained less than 3 inches in height throughout the season. These were in marked contrast with adjacent rows of Green Briar which ranged from 2 to 3 feet in height.

Microscopic Examination of Roots

Periodic microscopic examinations of roots of plants from non-treated plots were made during the summer. These revealed discolored root areas—the brown to black individual cells lacking any evidence of the presence of bacteria or fungi—and nematodes in various stages of development, all of which are so characteristic of the condition regarded as typical brown rootrot in Ontario. Immersion of affected roots in water overnight revealed the presence of a fair abundance of nematodes of different species. However, there was no correlation between the number of nematodes found and the severity of root injury.

Specimens of diseased roots submitted to G. Steiner confirmed the presence of meadow nematodes in them. Dr. Steiner states "the form involved is very close to one that is called the broad-headed nematode. It is not the type found in, for example, Florence, South Carolina, but it is present in Chatham, Virginia and Connecticut. In the material submitted they were not very numerous. We also found in these plants *Tylenchorlynchus claytoni.* This is a form that is continuously observed on dwarfed plants. We suspect this species to be very poisonous to the plant host. In Connecticut also this species is associated with plants of dwarfed appearance. In your instance, the invasion obviously was a very early one. Other parasitic nematodes found in these roots belong to the stem nematode, *Ditylenchus* sp."

SUMMARY

1. Six soil fumigants were applied to tobacco brown rootrot field soil and their relative effects on burley tobacco were compared.
2. Chemicals were applied in the row at different rates with a hand injector 10 days before planting.
3. Extremely wide differences in root injury and green weight yields were obtained between treated and non-treated plots of the susceptible variety Harrow Velvet.
4. The soil fumigants apparently acted as nematicides in the present experiments.

5. It is indicated that the variety Green Briar is much more tolerant to nematode populations in brown rootrot soil than most other varieties grown in Ontario.

6. Of the various fumigants used, Dow N, Chloropicrin, and Dow W40 resulted in greatly increased growth of the susceptible variety Harrow Velvet and thus appear to offer particular promise for the control of brown rootrot of tobacco.

ACKNOWLEDGMENT

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ARASAN AS A SEED AND SOIL TREATMENT FOR THE CONTROL OF DAMPING-OFF IN CERTAIN VEGETABLES

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INTRODUCTION

In southwestern Ontario, as in many other extensive vegetable-growing areas, damping-off of seedlings grown in flats or beds under greenhouse conditions is one of the most destructive diseases, and, until recently, many growers have accepted as unavoidable heavy losses in stands of young plants due to this trouble. In general, damping-off has been most severe where soil sterilization has not been practised. Nevertheless, in many large commercial houses, even when treated seed has been planted in steamed soil, appreciable losses have been sustained, particularly in stands of peppers, eggplant and Spanish onions. Moreover, when spot infections develop in flats of seedlings grown in steamed soil, the spread of the disease is usually more rapid, and the final loss is often greater than where non-steamed soil is used.

The annual losses suffered by growers, particularly those possessing no facilities for steaming seedbed soils, has warranted a search for improved control measures.

RECENT DEVELOPMENTS IN CONTROL OF DAMPING-OFF

In recent years, the wide adoption of seed treatment by most vegetable growers has closely followed the development of suitable seed protectants in dust form, several of which have shown a marked capacity to reduce pre-emergence damping-off. Especially noteworthy in this connection are the findings of Taylor and Rupert (7), who showed, in 1946, that seed treatment of spinach with Arasan is distinctly superior to treatment with Spergon and cuprous oxide in reducing post-emergence damping-off because of the more prolonged protective action of the first-mentioned material. More recently, a different approach to the control of certain soil-borne pathogens was developed at the Harrow laboratory by Hildebrand (4) and Hildebrand and co-workers (5), who showed that formulations of thiram (50 per cent non-wettable) when applied to the soil in the vicinity of sugar-beet seeds, effectively controlled both the pre-emergence and post-emergence phases of blackroot, a damping-off type of disease. Doran (1, 2), by a somewhat similar approach, has recently shown that damping-off of certain vegetables has been more effectively controlled by mixing certain organic fungicides with commercial fertilizers and applying them to the soil before seeding rather than in water after seeding.

Inasmuch as seed treatment with Arasan and several other of the newer fungicides failed to protect pepper seedlings adequately against post-emergence damping-off, the writer during the past three years has com-

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pared the effectiveness of seed and soil treatments with several fungicides for control of the disease in peppers and in certain other vegetables caused by *Pythium* spp. and *Rhizoctonia Solani* Kühn. In 1948 some preliminary results were reported (6). Further experiments and results are presented in this paper.

MATERIALS AND METHODS

The experiments were carried out in standard wooden flats 24″ × 12″ × 4″, under ordinary greenhouse conditions. Air temperatures varied from 60°–90° F. and, for the most part, closely approximated those obtaining in commercial greenhouses. In all experiments, the flats were sprinkled once or twice daily to maintain a moderately high soil moisture.

A compost mixture, designated type A, comprising Fox sandy loam soil, muck and well-rotted manure, was used in the first experiments reported in this paper. In later experiments another compost, type B, containing only Fox sandy loam soil and muck, was used. In the first-mentioned soil, damping-off organisms were abundant; but in the second it was necessary to add both *Pythium ultimum* and *Rhizoctonia Solani*, which were cultured on a cornmeal-sand medium. The inoculum was added to the soil 24–72 hours prior to seeding.

In preliminary trials, several of the newer fungicides were tested for control of damping-off in peppers. Of these, Arasan (50 per cent tetramethyl thiuram disulphide, non-wettable) gave consistently the best results and for this reason most of the experiments described below centred around the use of this chemical.

To ensure as uniform germination as possible in each test, small, light and off-type seeds were discarded. In spinach, the large seeds were not planted.

Seed treatment of these vegetables was carried out according to methods generally employed and precautions were taken to obtain uniformity in all tests. The dosage rates with Arasan were as follows: pepper—0.38 per cent, spinach—0.40 per cent, tomatoes—0.75 per cent, and 2.5 per cent, onion—0.24 per cent, cucumber—0.40 per cent, muskmelon—0.24 per cent, lettuce—2.0 per cent, and celery—2.8 per cent.

For soil treatment, the required amount of the protectant was uniformly mixed with the soil in the flat previous to planting. With the exception of preliminary tests with peppers, Arasan was applied to the soil at the rate of 3.14 gm. per cu. ft.

Uniformity of planting was obtained by seeding in depressed rows always of a fixed depth, 3/16 inches for small seeds, such as those of celery and lettuce, and 7/8 inches for large seeds, such as those of cucumber and muskmelons. After the seeds were evenly spaced in the row, the depression was filled, the seeds thereby being covered.

Counts of seedlings were made daily and those that damped-off were recorded and removed. The analysis of variance, as given by Hayes and Immer (3), was employed to determine significant differences between surviving stands of seedlings.

EXPERIMENTAL RESULTS

Arasan as a Protectant for Peppers

(a) Preliminary Tests

With the use of soil type A, preliminary tests had shown that 3.14 gm. of Arasan per cu. ft. of soil appeared to be effective in controlling the disease without being injurious to the host. Subsequently, the chemical was tested at double and triple this concentration. All soil treatments were checked against seed treatment alone and against a combined seed and soil treatment, five flats comprising the test unit in the various experiments. The results are summarized in Table 1.

As Table 1 indicates, all treatments resulted in considerably greater emergence than the check. Emergence was highest where Arasan was applied to the soil at the rates of 6.28 and 9.42 gm. per cu. ft. At these concentrations, however, subsequent growth of pepper seedlings was much retarded. No delay in emergence or retardation of later growth was apparent in the other treatments. The highest incidence of post-emergence damping-off, namely, 43.2 per cent occurred in the non-treated control. When seed alone was treated, the incidence of post-emergence damping-off was 35.2 per cent, indicating the relative ineffectiveness of seed treatment in controlling this phase of the disease. Soil treatment at the rate of 3.14 gm. allowed 14.6 per cent post-emergence damping-off and, when seed treatment was combined with soil treatment at the above-mentioned rate, post-emergence damping-off was reduced to 8.6 per cent, and the best stand of pepper seedlings was obtained.

TABLE 1.—EFFECT OF TREATMENT OF SEED, OF SOIL, AND OF SEED AND SOIL WITH ARASAN ON EMERGENCE AND DAMPING-OFF IN PEPPER SEEDLINGS

Arasan treatment		Toxicity effects	22 days after planting		
			Emergence* %	Post-emergence** damping-off %	Stand* %
Seed	(0.38%)†	None	87.1	35.2	56.6
Seed and Soil	(0.38% and 3.14 gm.)‡	None	93.0	8.6	84.7
Soil	(3.14 gm.)	None	87.0	14.6	74.6
“	(6.28 gm.)	Appreciable stunting	96.4	8.7	88.0
“	(9.42 gm.)	Marked stunting	96.6	2.1	94.6
None		None	76.8	43.2	43.6
Diff. for significance at 5%					9.7

* Throughout this study emergence and stand are expressed as percentages of the number of seedlings emerged and surviving, respectively, in relation to the number of seeds planted.

** Post-emergence damping-off is expressed as a percentage of affected seedlings in relation to emergence.

† By weight of seed.

‡ Per cu. ft. of soil.

The daily progress of seedling emergence and damping-off resulting from seed treatment, combined seed and soil treatment, and no treatment is shown graphically in Figure 1. This chart reveals the effectiveness of soil treatment in controlling post-emergence damping-off. The ineffectiveness of seed treatment alone in controlling this phase of the disease is shown by the similarity in slope of the curves for this treatment and the control.

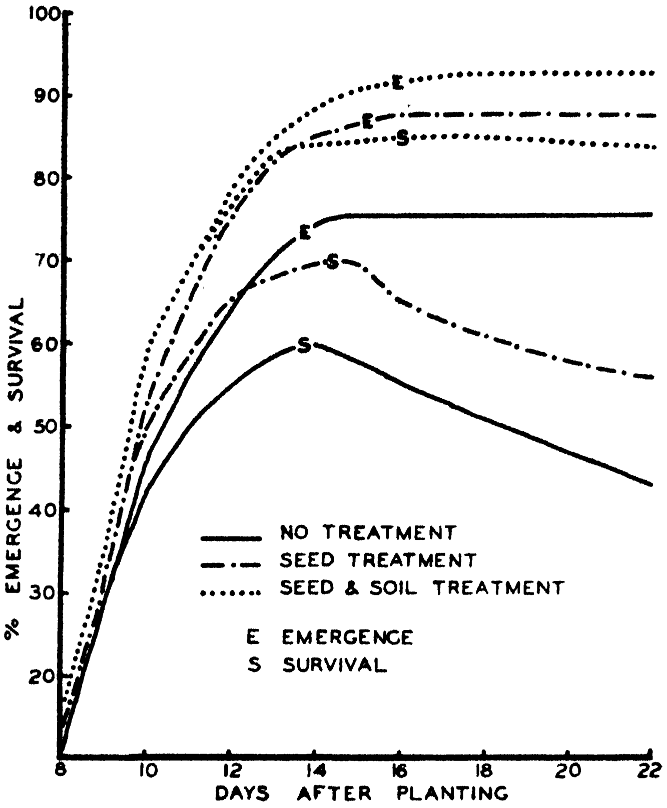

FIGURE 1. Emergence and survival of pepper seedlings as functions of time and treatment.

(*b*) *Confirmatory tests*

Following the preliminary trials described above, two additional similar experiments were conducted, one in the autumn and one in the spring. As the supply of naturally-infested compost soil type A was exhausted, type B was used in these experiments. The proportion of *Pythium* to *Rhizoctonia* in the inoculum added to this soil was in the ratio of 2 : 1, respectively. The results of the two tests being essentially similar, they were averaged and are presented in Table 2.

As Table 2 indicates, all treatments gave significantly better stands than the check. The combined seed and soil treatment proved to be superior to either seed or soil treatment alone and thus confirmed the results obtained in preliminary trials. In these tests, however, seed treatment was more effective in controlling the post-emergence phase of the disease than in the previous experiments. The reason for this difference is not evident. It should be mentioned, however, that, as a result of adding *P. ultimum* and *R. Solani* to the soil in the last two tests, the ratios of the damping-off pathogens would be somewhat different from those obtaining in non-inoculated soils. A photographic comparison between check and the combined seed and soil treatment is presented in Figure 2.

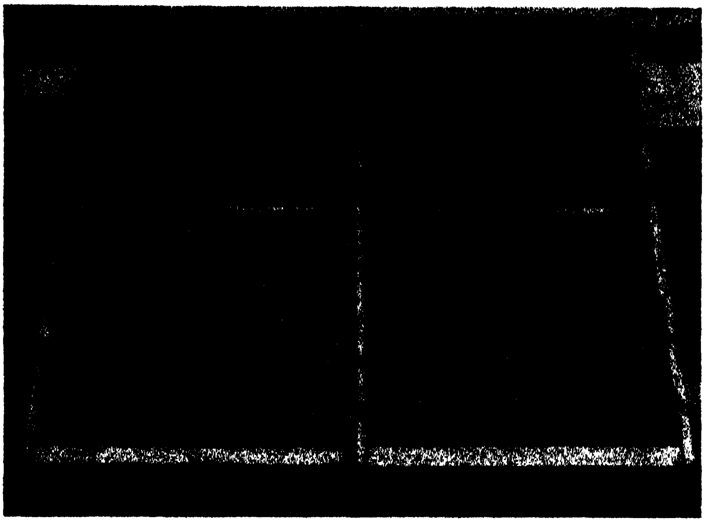

FIGURE 2. Effectiveness of Arasan seed and soil treatment in controlling damping-off in peppers. Treated seed planted in treated soil in upper half of both flats. No treatment in lower half of flats. Photograph taken 24 days after seeding.

Arasan as a Protectant for Other Vegetables

The highly satisfactory results obtained with peppers prompted similar tests with spinach, Spanish onions, lettuce, celery, cucumbers, muskmelons and tomatoes. Soil type B was used in these tests. The details and results of these experiments are summarized in Table 3.

With respect to cucumbers and muskmelons, Table 3 shows that all treatments produced highly significant increases in stand over the non-treated controls. No significant differences were evident between the seed or soil treatments with cucumbers. In muskmelons, however, the best stand of seedlings was obtained from the Arasan seed treatment alone. Post-emergence damping-off in muskmelons was appreciably greater in soil-treated flats than in either check or seed-treated ones. It should be

TABLE 2.—EFFECT OF TREATMENT OF SEED, OF SOIL AND OF BOTH SEED AND SOIL WITH ARASAN ON EMERGENCE AND DAMPING-OFF IN PEPPER SEEDLINGS

Arasan treatment		24 days after planting		
		Emergence* %	Post-emergence* damping-off %	Stand* %
Seed	(0.40%)	84.6	12.1	72.2
Soil	(3.14 gm.)	74.0	12.5	65.1
Seed and soil	(0.14% and 3.14 gm.)	88.5	5.9	84.2
None		65.8	29.1	48.2
Diff. required for significance at 5% level				9.5

* Average of 2 experiments, both containing 5 replications of 100 seeds each.

TABLE 3.—EFFECT OF TREATMENT OF SEED, OF SOIL, OF SEED AND SOIL WITH ARASAN ON EMERGENCE AND DAMPING-OFF IN CUCUMBERS, MUSKMELONS, SPANISH ONIONS, SPINACH AND TOMATOES

Host	Arasan treatment	Emergence* %	Post-emergence* damping-off %	Stand* %	Toxicity effects
			22 days after planting		
CUCUMBERS	Seed (0.40%)	92.0	0.0	92.0	Nil
	Soil**	92.4	2.6	90.0	Nil
	Seed and soil	96.4	2.0	94.4	Nil
	None	12.0	13.3	10.4	Nil
	Diff. for significance at 5%			5.27	
			23 days after planting		
MUSKMELONS	Seed (0.24%)	88.8	1.4	87.6	Nil
	Soil	79.6	15.1	68.0	Slight stunting
	Seed and soil	90.4	12.0	79.6	Slight stunting
	None	41.2	7.7	38.0	Nil
	Diff. for significance at 5%			11.51	
			29 days after planting		
SPANISH ONIONS	Seed (0.24%)	30.2	42.1	17.5	Nil
	Soil	75.5	15.2	66.5	Nil
	Seed and soil	82.5	10.0	74.2	Nil
	None	20.5	54.8	9.2	Nil
	Diff. for significance at 5%			6.70	
			23 days after planting		
SPINACH***	Seed (0.40%)	22.4	23.7	17.1	Nil
	Soil	43.3	9.0	39.1	Slight stunting
	Seed and soil	50.4	10.5	45.1	Slight stunting
	None	17.2	27.1	11.4	Nil
	Diff. for significance at 5%			5.63	
			28 days after planting		
TOMATOES	Seed (0.75%)	89.4	2.1	87.5	Nil
	Soil	75.6	7.9	69.8	Slight delay in germination
	Seed and soil	85.6	2.2	83.8	
	None	65.1	23.4	49.9	Nil
	Diff. for significance at 5%			9.10	

* These results are averages of 5 replications of 100 seeds each for all treatments for onions, spinach and tomatoes, and of 50 seeds each for cucumbers and muskmelons.

** Arasan was applied at the rate of 3.14 gm. per cu. ft. of soil in all soil treatments referred to in this table.

*** The germinating capacity of the spinach seed used in these tests was approximately 58%.

mentioned that this phase of the disease did not develop in soil treatments until the seventh day after emergence. Further evidence that Arasan, when applied to the soil, was slightly injurious to young muskmelon plants and thereby seemingly rendered them more susceptible to attack, was indicated by a conspicuous retardation in early growth of seedlings.

When isolations were made from 12 muskmelon seedlings that damped-off in soil-treated flats, the cultures obtained yielded 11 of *Rhizoctonia* and one of *Pythium*, indicating that the former fungus was predominantly responsible for the disease at that stage.

If reference is made to Table 3 again, it is apparent that seed treatment afforded some small measure of protection to onion seedlings during the early stages of growth, as evidenced by an increase in emergence from the treated seed over the check. That seed treatment provided protection for only a short period of time was demonstrated by the relatively low emergence and the high incidence of post-emergence damping-off. Soil treatment alone and the combined seed and soil treatments were distinctly more effective than seed treatment in controlling both phases of the disease, as they gave much higher emergence and lower incidence of post-emergence damping-off. At the termination of the experiment, the stand of onion seedlings from soil treatment was almost four times greater than that obtained from seed treatment.

For the control of damping-off in spinach, the superiority of soil treatment over seed treatment is readily apparent. The former not only permitted much higher emergence but also controlled post-emergence damping-off more effectively than the latter. Moreover, a combined seed and soil treatment controlled the pre-emergence and post-emergence phases of the disease more effectively than soil treatment alone. Mention should be made, however, that applications of Arasan to the soil caused a slight retardation in the early growth of spinach seedlings, especially under conditions conducive to rapid growth.

For tomatoes, all treatments were significantly superior to the check. Seed treatment alone gave slightly higher emergence and final stands than the combined seed and soil treatment. Soil treatment alone was inferior to seed treatment in respect to the protection afforded against pre-emergence and post-emergence phases of damping-off. There was also observed a significant retardation in emergence of seedlings to the extent of 12–36 hours when Arasan was applied to the soil. Moreover, it was demonstrated in earlier trials that a similar delay could be obtained by applying heavy dosages to the seed.

The data concerning tests with celery and lettuce are not presented as the incidence of damping-off was too low to indicate the value of treatments. It was observed, however, that seedling emergence in these two vegetables was delayed and reduced by the Arasan treatments, thus indicating appreciable toxicity of the chemical to these crops.

Comparative Effectiveness of Arasan and Formaldehyde Treatments in Controlling Damping-off in Tomatoes

As some growers in southern Ontario have considered the treating of soil with formaldehyde prior to planting essential to the production of uniformly good stands of tomato seedlings, a comparison between Arasan and formaldehyde treatments for disease control seemed desirable. For this experiment formaldehyde dust was applied to the soil at the rate of 215 gm. per cu. ft. The Arasan treatments were the same as those in the previous test. Four weeks after planting, all seedlings were removed from the flats. They were then washed free from soil, and the roots and hypocotyl were examined for presence of brownish lesions caused by *Rhizoctonia*. The results of the experiment are summarized in Table 4.

An examination of Table 4 reveals that all treatments were effective in controlling damping-off. It was observed that the emergence was slower in the Arasan treatments than with the formaldehyde treatment, but the

TABLE 4.—COMPARATIVE EFFECTIVENESS OF ARASAN AND FORMALDEHYDE TREATMENTS IN CONTROLLING DAMPING-OFF OF TOMATOES

Treatment		Percentage* of plants 28 days after planting			
		Emergence	Post-emergence damping-off	Stand	Lesioned**
Arasan on seed	(2.5%)	96.7	0.0	96.7	32.8
Arasan in soil	(3.14 gm.)	91.7	0.3	91.5	40.6
Arasan on seed and in soil	(2.5% and 3.14 gm.)	96.2	0.0	96.2	7.4
Formaldehyde dust in soil	(215 gm.)	97.5	0.3	97.2	9.2
No treatment		53.5	2.8	52.0	65.8

* Average of 2 replications of 200 seeds each.

** These values represent the percentage of surviving plants exhibiting prominent *Rhizoctonia* lesions on either the lower part of the hypocotyl or upper region of the primary root.

final emergence was almost the same. The initial advantage gained from the earlier emergence in the formaldehyde-treated soil was retained and manifested in subsequent above-ground growth of the plants. In the Arasan treatments, however, the young plants were darker green in colour and were sturdier than those growing in the formaldehyde-treated soil. From an examination of seedlings for presence of lesions caused by *Rhizoctonia*, it was found that disease control was approximately the same in the combined Arasan seed and soil treatment as in the formaldehyde treatment. These two treatments were markedly more effective than either the Arasan seed or soil treatment alone.

DISCUSSION OF RESULTS

For many years, much research has been aimed at finding a suitable seed treatment that would not only disinfect the seed surface but would also protect the seedlings during the entire period of susceptibility to damping-off pathogens. In the light of the foregoing results, it would seem that in the case of such vegetables as onions and spinach, which are highly susceptible to damping-off both before and after emergence, seed treatment with Arasan falls far short of providing the requisite protection. Furthermore, a crop very susceptible to damping-off for a long period after emergence, such as peppers, requires more than a protective seed treatment. From the results of the above-described experiments, it would appear that seed treatment with Arasan furnishes the germinating seed with immediate protection and that the soil treatment affords the seedling additional protection after it has emerged beyond the sphere of influence of the chemical. It is suggested that the efficacy of seed treatment on certain large-seeded vegetables, such as cucumbers and muskmelons, might be explained by the influence of the chemical radiating over a sufficiently large area of soil to protect the seedling for the required period. Similarly, the lack of control afforded by seed treatment on certain small-seeded vegetables, such as onions and spinach, might be due to inadequate spatial protection.

Due to the suppression of attack by *Rhizoctonia* through soil treatment with Arasan for a period of time rather than the complete elimination of the pathogen, the advisability of treating all the soil in the flat may be questioned.* Hildebrand *et al.* (5) showed that the incidence of blackroot in sugar beets was negligible if the seedlings grew up through soil treated with Arasan. Similarly, it may well be that, in vegetables, adequate protection may be obtained by treating only the soil covering the seed. Moreover, the possibility exists that toxicity to Arasan may be reduced in such hosts as muskmelons, lettuce and celery by treating only a limited amount of soil in the flat. Further experiments will be necessary to decide this issue.

Although soil treatment may have the slight disadvantage of increasing the amount of labour required at seeding time, nevertheless, the benefits to be derived from the treatment, where it may be used, would appear to more than offset the former. The treatment may be regarded as of particular benefit to vegetable growers who possess no facilities for steam sterilization of soils.

SUMMARY

A comparative study of the effectiveness of seed treatment, of soil treatment, and of a combined seed and soil treatment with Arasan and certain other chemicals was conducted in an effort to control damping-off of peppers, tomatoes, spinach, Spanish onions, cucumbers, muskmelons, lettuce and celery.

Damping-off of spinach and Spanish onions was more effectively controlled by soil treatment with Arasan at the rate of 3.14 gm. per cu. ft. of soil than by seed treatment, but a combined seed and soil treatment with Arasan was better than soil treatment alone.

Significantly better stands of pepper seedlings were obtained from use of the combined treatment than from either one alone; soil treatment was effective in controlling the post-emergence phase of the disease for a longer time than was seed treatment.

In tomatoes, seed treatment with Arasan gave effective control of damping-off in the seedbed. The combined Arasan treatment was as effective in disease control as was the widely used formaldehyde soil treatment, but the former usually retarded emergence by a few hours.

By seed treatment, cucumbers and muskmelons were afforded a high degree of protection. In soil treated with Arasan, the incidence of post-emergence damping-off in muskmelons was higher than in the non-treated control, indicating that the chemical was injurious to this vegetable when applied to the soil.

Arasan, when applied to either the seed or the soil, was toxic to both lettuce and celery, as indicated by delayed and reduced emergence.

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The author wishes to express his gratitude to L. W. Koch and A. A. Hildebrand, of the Harrow laboratory, for helpful criticism of the manuscript.

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* This statement is based upon more recent experiments, the results of which will be made known in a later publication.

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DIGESTIBILITY STUDIES WITH FOXES

III. DIGESTIBILITY OF SOME FOX FEEDS AND FOX RATIONS[1]

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Two previous papers from this Station (4, 5) have reported the digestibility coefficients of several meat products commonly fed to foxes in Canada. In another paper Inman (3) reported the digestibility coefficients of a number of commercial fox feeds. However, since this report was published, many changes have been made in these mixed feeds, both in the ingredients used and in their percentage composition. Therefore further digestibility trials on these newer rations were carried out during the winter of 1948-49.

This paper reports the chemical composition and digestibility coefficients of a number of meat by-products, vegetables, complete fox rations and also a few commercial fox feeds commonly used by Canadian fox ranchers.

PROCEDURE

Adult male foxes (over one year of age) were used in all cases except when pup rations were fed and then approximately 10-months-old male pups were used. Each of the feeds and rations reported in this paper were fed to at least four different foxes and the average digestibility coefficient calculated.

All feeds were fed in the ground state. Beef manifolds, fox carcasses, whalemeat, and the four rations containing raw and cooked cereal were fed alone at the rate of 300 gm. per fox each day. All other feeds tested were fed in combination with horse-meat and their digestibility coefficients calculated by difference. Blood meal, ground green bone, bone meal, the two fish meals, meat meal, and the eleven commercial mixed feeds were fed at the rate of 50 gm. per day together with 150 gm. of ground horse-meat. The cabbage, carrots, turnips, and frozen fish rations were fed at the rate of 150 gm. to 150 gm. of horse-meat while the canned tomatoes were fed at the rate of 190 gm. to 150 gm. of ground horse-meat. Where necessary, sufficient water was added to the mixture to bring it to a "hamburger" consistency. These quantities were readily consumed by the foxes used on the feeding trials. Each day's ration for the entire feeding trial was weighed, wrapped in wax paper and frozen until the day it was to be fed.

Each trial was of seven-day duration. On the second day 0.5 gm. of ferric oxide was added to the ration as recommended by Bell and Crampton (2) and this marked feces was collected. On the seventh day 0.5 gm. of ferric oxide was again added to the ration and the marked feces was discarded. Thus there were five days' feces collected. The metabolism cages used in these feeding trials and the method of preserving the feces were the same as used in previous trials (4, 5).

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TABLE 1.—CHEMICAL COMPOSITION OF FOX FEEDS AND RATIONS AVAILABLE IN CANADA

	Dry matter	Protein (N × 6.25)	Crude fat	Crude fibre
	%	%	%	%
Beef manifolds	21.6	11.6	8.9	—
Blood meal	89.4	79.2	1.3	—
Bone, ground green	60.3	21.2	14.4	—
Bone meal, steamed	92.3	25.4	5.1	—
Cabbage, fresh	7.7	1.5	0.5	1.2
Carrots, fresh	12.0	1.2	0.3	1.1
Fish, frozen (Novascot fishblox)	23.5	16.1	1.3	—
Fish meal (low fat)	90.6	65.1	2.9	—
Fish meal (high fat)	89.6	49.9	13.2	—
Fox carcasses	43.4	19.2	20.7	—
Horse-meat (average analysis from all trials)	28.2	19.7	6.5	—
Meat meal	93.4	48.1	15.1	—
Tomatoes, canned	5.7	0.9	0.5	0.6
Turnips, fresh	9.3	1.6	0.5	0.9
Whale meat	32.4	26.9	3.6	—
Adult ration containing raw cereal	43.1	15.2	2.8	1.8
Adult ration containing cooked cereal	42.5	15.3	2.5	1.6
Pup ration containing raw cereal	41.0	13.4	5.8	1.4
Pup ration containing cooked cereal	42.4	13.4	6.8	1.4
Hexite cereal fox meal	89.8	14.3	4.4	4.2
Hexite XX fox meal	91.9	26.7	5.6	4.3
Master pelting ration	95.8	18.6	4.2	4.5
Master breeding ration	90.1	19.8	4.1	3.8
Master fox puppy starter	91.5	20.9	5.2	4.3
Miracle cereal ration	89.3	14.6	4.2	5.0
Miracle fox meal	89.4	27.3	5.5	4.4
Purina fox chow	87.9	25.9	4.5	4.5
Ross-Miller standard cubes B	87.6	19.0	4.7	3.7
Ross-Miller kibblo	91.9	19.1	0.9	2.4
Ross-Miller fox crisps	89.3	19.7	1.0	2.1

The analyses for dry matter, crude fat, and crude fibre were conducted according to the methods of the A.O.A.C. (1). The procedure for protein (N × 6.25) analysis was similar to the A.O.A.C., except that 50 ml. of 4 per cent boric acid were placed in the receiver and a standard sulphuric acid solution was used for titrating.

RESULTS AND DISCUSSION

Table 1 shows the chemical compositions of the individual feeds and rations used in the digestibility trials, while Table 2 presents the average digestibility coefficients of these same foodstuffs. The coefficients of digestibility for horse-meat shown in Table 2 are the averages of 28 digestibility trials.

It will be noted that the digestibility coefficients of the protein of cabbage and carrots exceeded 100 per cent. This no doubt is accounted for by the fact that these two foodstuffs of low protein content were fed in combination with a high protein feed (horse-meat), and any slight variation in the digestibility of the protein of horse-meat would be greatly increased when the difference was transferred to the digestibility of the low protein feeds. This fact would also account for the low digestibility of the fat of Ross-Miller "Kibblo" and Ross-Miller "Fox Crisps."

TABLE 2.—DIGESTIBILITY COEFFICIENTS OF FOX FEEDS AND RATIONS AVAILABLE IN CANADA

	Dry matter	Protein (N × 6.25)	Crude fat	Crude fibre
	%	%	%	%
Beef manifolds	91 ± 1.0	93 ± 1.0	96 ± 0.1	—
Blood meal	55 ± 4.0	62 ± 4.0	—	—
Bone, ground green	41 ± 4.0	66 ± 4.0	99 ± 1.0	—
Bone meal, steamed	15 ± 6.0	40 ± 4.0	88 ± 6.0	—
Cabbage, fresh	74 ± 2.0	100 ± 8.0	50 ± 8.0	43 ± 5
Carrots, fresh	83 ± 3.0	104 ± 9.0	66 ± 5.0	32 ± 11
Fish, frozen (Novascot fishblox)	76 ± 5.0	95 ± 1.0	71 ± 4.0	—
Fish meal (low fat)	75 ± 2.0	91 ± 2.0	63 ± 7.0	—
Fish meal (high fat)	61 ± 3.0	69 ± 5.0	94 ± 1.0	—
Fox carcasses	84 ± 0.4	92 ± 1.0	99 ± 0.1	—
Horse-meat	94 ± 1.0	94 ± 1.0	97 ± 1.0	—
Meat meal	61 ± 5.0	80 ± 3.0	92 ± 2.0	—
Tomatoes, canned	81 ± 2.0	92 ± 9.0	45 ± 5.0	54 ± 6
Turnips, fresh	31 ± 6.0	69 ± 17.0	66 ± 5.0	33 ± 14
Whale meat	90 ± 2.0	97 ± 1.0	95 ± 2.0	—
Adult ration containing raw cereal	76 ± 1.0	87 ± 1.0	90 ± 1.0	15 ± 7
Adult ration containing cooked cereal	80 ± 1.0	83 ± 0.3	85 ± 1.0	16 ± 7
Pup ration containing raw cereal	66 ± 3.0	82 ± 2.0	92 ± 1.0	8 ± 7
Pup ration containing cooked cereal	70 ± 0.5	79 ± 1.0	93 ± 0.5	15 ± 6
Hexite cereal fox meal	74 ± 2.0	79 ± 3.0	75 ± 2.0	15 ± 8
Hexite XX fox meal	66 ± 2.0	80 ± 3.0	77 ± 2.0	24 ± 9
Master pelting ration	70 ± 4.0	73 ± 6.0	59 ± 6.0	20 ± 12
Master breeding ration	71 ± 6.0	82 ± 6.0	78 ± 3.0	7 ± 14
Master fox puppy starter	69 ± 2.0	81 ± 2.0	74 ± 2.0	19 ± 3
Miracle cereal ration	76 ± 6.0	85 ± 1.0	74 ± 8.0	6 ± 2
Miracle fox meal	73 ± 1.0	85 ± 2.0	79 ± 3.0	12 ± 4
Purina fox chow	71 ± 0.2	78 ± 0.2	74 ± 2.0	10 ± 6
Ross-Miller standard cubes B	71 ± 3.0	84 ± 3.0	74 ± 3.0	3 ± 3
Ross-Miller kibblo	84 ± 2.0	87 ± 2.0	1 ± 1.0	8 ± 8
Ross-Miller fox crisps	86 ± 2.0	88 ± 3.0	27 ± 10.0	21 ± 4

Cooking of the cereal portion of the adult fox ration and the pup ration increased the total digestibility of the rations by approximately 4 per cent. The cereal portion of these rations consisted of equal parts of rice, wheat, oats, barley and corn.

The digestibility of crude fibre was in general quite low, but was extremely variable.

SUMMARY

The chemical analysis and coefficients of digestibility have been reported for 14 feeds, 4 complete rations, and 11 commercial fox feeds commonly fed by Canadian fox ranchers.

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STUDIES OF COMBINING ABILITY IN BROMEGRASS AND CRESTED WHEATGRASS[1]

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The recognition of combining ability as an important factor in the selection of plants and inbred lines in corn has stimulated considerable interest in the possibilities of selecting other cross-pollinated crops upon a similar basis. Studies of combining ability have been quite extensive in alfalfa and breeders of this crop have suggested methods by which plants with good combining ability may be used to produce high yielding synthetic varieties and commercial hybrids. Selection in perennial forage grasses on the basis of combining ability is being practised at plant breeding stations in North America at the present time but as yet there is little published evidence of the success of selection on this basis or of the breeding techniques by which combinin[illegible]ility may be evaluated.

Investigations were undertaken at Saskatoon in bromegrass and crested wheatgrass to assess the range of interplant variation in combining ability and to compare the reliability of various kinds of progenies which might be used in determining the combining ability of individual plants. Data were analysed to show the relationship of specific combining ability, or the response of plants in particular crosses, to general combining ability, or the average response of plants in crosses. Correlations of the performances of parent plants and progeny classes also were made to provide indications of the heritability of yield and other characters.

LITERATURE REVIEW

The use of inbred lines in breeding bromegrass was reported by Kirk (14, 15), Popravko (22), Stevenson (26), Hayes and Schmid (9), and Tsiang (29). The species was found to be largely self-sterile yet containing partially self-fertile plants in sufficient numbers to permit the production of selfed lines. Selfing caused reductions in vigour and fertility of lines and consequent difficulties in carrying them to advanced generations of inbreeding. Hayes and Schmid (9) produced a limited number of single crosses which outyielded the commercial variety.

Hill and Myers (10) reviewed the literature on chromosome numbers in bromegrass and reported an extensive study of strains of various origins. They found a chromosome number of 56 (2n) for 192 of 193 plants studied. The single variant had 8 to 11 accessory chromosome fragments in addition to the regular complement. Elliott and Love (5) found marked irregularity in the meiotic behaviour of bromegrass and suggested that non-random selection of gametes might be anticipated. Elliott and Wilsie (6) observed good fertility and regularly normal bivalent association at meiosis in a haploid plant. This they regarded as evidence of an autopolyploid derivation for the species.

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The nomenclature recommended by Dillman (4) has been accepted by the present writer in discussing the Fairway and Standard types of crested wheatgrass. The shorter growing, finer stemmed, and more leafy Fairway type was classified by Dillman as *Agropyron cristatum* (L.) Gaertn., and the taller growing, coarser Standard type as *A. desertorum* (Fisch.) Schult. Dillman found the Fairway and Standard types to have 2n chromosome numbers of 14 to 28, respectively. This is in agreement with the results of Peto (21), Myers and Hill (19), and Murphy (18) but these authors also observed aneuploid plants with from one to three chromosomes in addition to the normal number in the Standard type. Myers and Hill considered the Standard type to be an autopolyploid in view of the high degree of quadrivalent association of chromosomes at meiosis. Murphy (18), and Cheng (2) indicated that both types were sufficiently self-sterile to prevent selfed line methods of breeding. Stevenson (27) indicated the unsuitability of inbreeding techniques in crested wheatgrass as inbred lines showed marked loss of vigour.

Extensive studies of combining ability, or the ability of plants or lines to transmit desirable qualities to their crosses, were undertaken in corn coincident with the development of hybrid programmes. Jenkins and Brunson (11) found that combining ability as indicated by single crosses was closely correlated with combining ability as shown in inbred-variety crosses. To expedite the testing of lines Sprague (24) advocated testing in early generations even to the extent of testing original non-inbred (So) plants. Evidence of the heritability of combining ability in corn has been given by Sprague (24), Johnson and Hayes (12), Cowan (3), and Green (8). Sprague and Tatum (25) described "general combining ability" as the average performance of lines in hybrid combinations and distinguished it from "specific combining ability" or the ability of lines to perform relatively better or more poorly in crosses than would be expected on the basis of average performance. Green (7) and Keller (13) showed that specificity of combining ability was exhibited by other than inbred material. When single F_2 plants were tested in top-crosses to tester hybrids and varieties there was significant variation in the order of F_2 plants for combining ability depending on the tester used. Robinson *et al.* (23) studied character heritability in corn crosses by analysis of variance methods and progeny-parent regressions. Heritabilities for various morphological characters such as plant height and ear height were notably higher than that for yield.

Tysdal and associates in Nebraska (30, 31, 32) and Bolton and Stevenson in Canada (1, 28) applied combining ability concept to the breeding of alfalfa. In particular, they developed breeding techniques by which plants with high combining ability suitable for inclusion in strain building plots may be identified. While single crosses were used for critical tests of the combining ability of single plants Tysdal *et al.* (30) also proposed polycross nurseries to obtain seed of a large number of selected plants simultaneously. In this method plants or lines to be tested were placed together in isolated nurseries and allowed to interpollinate. Replication and randomization of plants or lines in the polycross were suggested to insure random pollination and to provide a bulk of seed sufficient for regional tests.

Tysdal and Crandall (32) compared the combining ability rating of seven plants of alfalfa as shown by polycross yields with the rating as indicated by the average yields of three to five single crosses. Good correspondence was found. They also obtained good agreement between polycross progeny yields and top-cross yields where the top-cross was made between each clone and a commercial variety. Fair agreement only was indicated for the comparison of polycross progeny yields and the yields of modified top-crosses produced by growing the commercial variety in alternate rows between rows of the selections. Bolton (1) investigated the use of single clones as testers for other selected plants. The yields of tester-crosses when compared with the average yields of single crosses gave correlation coefficients of + 0.657 and + 0.671 for yields of seed and +0.644 and + 0.939 for yields of forage. The use of high yielding plants as testers gave similar results to those from using low yielding plants. Increasing the number of tester plants from one to two or to four did not materially increase the efficiency of the test for combining ability.

The results of Tysdal and Crandall (32) indicated that marked breeding progress could be achieved by the selection of alfalfa plants with high combining ability. Eight synthetics were found to give yields in accordance with those predicted from combining ability tests of plants constituting them. The highest yielding synthetic yielded 116 per cent of the commercial variety and the average polycross progeny yields of parent plants entering the synthetic was 111 per cent of the commercial variety.

MATERIALS AND METHODS

Studies of combining ability were conducted in bromegrass, *Bromus inermis* Leyss., crested wheatgrass of the Fairway type, *Agropyron cristatum* (L.) Gaertn., and crested wheatgrass of the Standard type, *A. desertorum* (Fisch.) Schult. In each species the method of study was to intercross a small number of selected plants in all single cross combinations and relate the performance of resultant progenies with that of progenies derived from open-pollination and polycrosses and with the parental clones. Yield of forage was the main consideration throughout all comparisons but since bromegrass is a rhizomatous species observations also were made in this grass of the degree of creeping. In the Standard type of crested wheatgrass an infestation of the western wheat aphis (*Brachycolus tritici* Gill.) in progeny nurseries allowed the observation of strain resistance to this pest.

Plant Materials

Single crosses in bromegrass were made among seven plants of the Superior variety. This strain is similar to the northern commercial type of bromegrass, differing from it mainly in being less strongly creeping. Plants used in this study had no other previous breeding history and were selected in space-planted nurseries as having superior height, leafiness, and resistance to leaf-spot diseases. Selection also was based upon high self-sterility but, as will be shown later, plants were less self-sterile than preliminary tests had indicated. The plants were selected from an original population of 266 thus giving a selection intensity of 2.6 per cent.

Single crosses in the Fairway type of crested wheatgrass were made within one group of nine plants which had no previous breeding history and a second group of seven plants which had been inbred. Inbred plants consisted of two plants from each of inbred lines A, C, and D, and one from inbred line B. These lines had been inbred for one generation except for line A which had been inbred for three generations. Crosses in the Standard type of crested wheatgrass were made among seven plants of a commercial lot. All plants of crested wheatgrass were selected from single plant nurseries as having good height, leafiness and vigour, and also a high degree of self-sterility as shown in preliminary tests. The intensity of selection for the non-inbred group of Fairway material was 7 per cent and for both the Fairway and the Standard type plants the intensity of selection was 10 per cent.

The methods of producing crossed seed for these studies were similar to those described by Knowles and Horner (16) for crested wheatgrass. These consisted essentially of the bulk transference of pollen between highly self-sterile plants without any emasculation. Bromegrass crosses were made mainly by the method of bag-switching, that is, an exchange of pollination bags between pairs of plants when flowering was in progress. Crested wheatgrass crosses were made by bag-switching and by the mutual pollination of clones planted together in paired combinations. Open-pollination progenies were grown from seed obtained from the original selections following natural pollination in the field. In bromegrass such seed was harvested from clonal rows of a reserve nursery containing a total of 120 clonal lines. This nursery contained two replications with clones randomized in each replication. Progenies grown from seed of clones in the first replication of this nursery will be referred to as "Open-pollination progenies Source-1", and from the second replication as "Open-pollination progenies Source-2". Open-pollination seed of crested wheatgrass was collected from the original selected plants growing in observation nurseries at a spacing of 3 ft. × 3 ft.

Polycross seed of bromegrass was obtained from an isolated plot in which the seven selected plants and three additional plants were each represented by four separate clones. Clones were arranged in the polycross plot in four replicates with a clone of each of the ten strains being placed at random in each replicate block. Polycross seed was not obtained for crested wheatgrass but top-crossed seed was available for five of the nine plants of the Fairway group. Pollinations in this case were made with the pollen gun using bulk pollen collected from 20 random plants of the Fairway variety.

Establishment of Field Trials

All material was tested under space-planted conditions in the field. Seedlings and clones were started in the greenhouse and transplanted to the field in June 1947. Clonal establishment was less successful than seedling establishment particularly in crested wheat grass. Each of the four groups of controlled crosses and related progenies was grown in a separate yield test. Triple lattice repeated designs with six replications were used in all trials. The tests of bromegrass and of non-inbred Fairway crested wheatgrass each included a total of 64 strains while the tests of inbred Fairway and Standard crested wheatgrass contained 49 entries. Plots consisted of single rows 16 to 20 feet in length with rows spaced three feet apart from bromegrass and two feet apart for crested wheatgrass. Plant spacing within the rows was two feet except in the test of the Standard type of crested wheatgrass where a spacing of one and one-half feet was used. In each trial all possible single crosses between parent plants were represented and in three of the four groups of plants several reciprocal single crosses were present.

Check strains in the test of bromegrass included the Superior variety, northern commercial bromegrass purchased from a local seed house, the Achenbach variety obtained from Kansas State College, Manhattan, Kansas and the Parkland variety maintained at the Dominion Forage Crops Laboratory, Saskatoon. Checks in tests of the Fairway type of crested wheatgrass consisted of the Fairway variety and strains S-39-$\bar{3}$-1, S-38-$\bar{9}$-$\bar{1}$-3, S-39-$\bar{7}$-1, and S-32-$\overline{28}$-1-1 each developed by the isolated increase of lines originally derived from inbreeding. Check lots in the test of the Standard type of crested wheatgrass were S-1333, S-131, Standard and Forage. Strains S-1333 and S-131 represent preliminary strains of the Standard type developed by the increase of introductions from the United States Department of Agriculture and the U.S.S.R., respectively. The Standard strain was obtained from the United States Department of Agriculture and traced to the introduction S.P.I. 19537. The Forage strain was developed at the Dominion Forage Crops Laboratory by the increase of a single plant selection. This strain was rather extensively distributed in Saskatchewan in 1935 and 1936 but never licensed as a variety. It is probable that much of the commercial seed of the Standard type produced in Saskatchewan to-day traces to this source.

Nature of Observations

Yields of hay in terms of green weight, and other observational data were obtained in 1948. The percentages of original plants that were missing at harvest-time were also noted and found to be respectively 6.0, 2.6, 10.0 and 3.5 for tests of bromegrass, non-inbred Fairway crested wheatgrass, inbred Fairway crested wheatgrass, and Standard crested wheatgrass. Yields per plot were corrected for the number of missing plants by direct linear corrections. For example, the harvested yields of 11-plant plots were multiplied by the factor 11/10 when one plant was missing. In bromegrass the degree of creeping was determined by measuring the diameter of spread of three plants in each plot. These measurements were made immediately after plots were cut for hay in the second year of growth. In view of the minor degree of leaf spot infection in bromegrass in 1948 it was not possible to compare strains in regard to disease resistance. In 1948 all parent plants involved in these studies were retested for self-sterility by placing 12 selfing bags on clones of each plant.

TABLE 1.—AVERAGE YIELDS IN POUNDS GREEN WEIGHT PER PLOT OF SINGLE CROSSES AMONG SEVEN SELECTED PLANTS OF BROMEGRASS COMPARED TO THE YIELDS OF OPEN-POLLINATION PROGENIES, POLYCROSS PROGENIES, PARENT CLONES, AND CHECK VARIETIES (SIX-REPLICATE TEST OF 64 ENTRIES, PLANTED, JUNE 1947, HARVESTED, JULY 1948, YIELDS CORRECTED BY COVARIANCE FOR DEGREE OF CREEPING)

Selection	Single cross when selection in column one crossed with:						Average of single crosses	Open-pollination progeny		Polycross progeny	Parent clone
	18	19	83	85	87	90		Source-1	Source-2		
16	— 11.5	11.6 —	12.2* 12.4	11.2 9.1	9.5 10.8	13.1 12.6	11.8	12.2	13.0	12.6	9.5
18		— 12.5	10.0 11.2	11.7 9.1	12.4 —	11.3 12.9	12.0	11.6	12.3	12.8	11.1
19			12.4 12.0	12.1 —	— 10.0	12.1 —	11.8	12.5	11.8	12.4	9.4
83				10.2 9.4	11.7 12.0	9.0 —	11.2	11.9	11.4	12.9	10.9
85					9.4 —	9.8 14.3	11.5	12.9	—	12.6	9.2
87						11.8 —	11.1	12.1	12.2	13.0	11.7
90							12.2	13.8	13.8	12.9	13.3
Range							1.1	2.2	—	0.6	4.1
Average							11.7	12.4	—	12.7	10.7
Average all crosses and progenies							11.8				
Checks—Superior							12.7				
Commercial (1)							12.5				
Commercial (2)							12.8				
Parkland							10.2				
Achenbach							13.1				
Av. checks							12.3				
							Difference in creeping of mean				
							0 in.	4 in.	8 in.		
L.S.D. (P = 0.05)—means of 6 plots							1.5	1.5	1.6		
means of 36 plots							0.6	0.6	0.7		
means of 6 and 36 plots							1.2	—	—		

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* The upper value in each cell indicates the yield of the cross when the selection in column one was used as the female parent, i.e. 16 × 83. The lower value in each cell indicates the yield of the reciprocal cross, or 83 × 16.

Differential damage to strains of the Standard type of crested wheatgrass from infestations of the western wheat aphis (*Brachycolus tritici* Gill.) was noted in the spring of 1948. Damage consisted of a marked dwarfing of plants similar to that caused by this same pest in winter wheat (20). Negligible effects were noted in the Fairway type of crested wheatgrass and no damage of any kind in bromegrass. Plots within the test of intercrosses of the Standard type were scored from 1 to 10 for resistance to damage with a score of 1 representing very severe effects and a score of 10 no injury.

EXPERIMENTAL RESULTS

Bromegrass

Analyses of variance for the test of bromegrass material indicated highly significant differences among the 64 strains entering the test both for yield of forage and for degree of creeping. The coefficients of variability in these analyses were 12 per cent with respect to yield and 17 per cent with regard to the degree of creeping. Analyses of variance as for a lattice design resulted in a gain in efficiency of 11 per cent in testing for yield and no gain in efficiency in testing for degree of creeping. A significant relationship ($r = +0.63$) was found between yield and the degree of creeping and yields were corrected for the degree of creeping by covariance methods. Since small gains in efficiency were obtained by using lattice methods of analysis, the method of analysis as for a randomized block design was used throughout for variance and covariance determinations.

Hay yield data are presented in Table 1 and the degree of creeping data in Table 2. Least significant differences based on means of 6 plots are given for the comparison of individual strains, and on means of 36 plots for the comparison of the average response of single crosses for different selections. Least significant yield differences also are noted where strains differ in degree of creeping. Least significant differences based on means of 6 and 36 plots are included for the comparison of individual strains and averages of single crosses. A subdivision of strain variance according to types of progenies and clones in the test has been provided in Table 3.

Data in Table 1 indicate considerable variation in the yields of reciprocal crosses. Poor correspondence of reciprocals apparently resulted from partial self-fertility of certain selections and the inclusion of selfed plants within some of the single crosses. In tests of self-fertility in 1948 it was found that selections 85 and 83 produced 22.1 and 12.8 seeds per panicle, respectively when selfed whereas other selections when self-pollinated gave fewer than three seeds per panicle. In progenies of crosses between plants 16 and 85, 18 and 85, and 90 and 85 there were significant differences between the yields of reciprocal crosses with the low yielding reciprocal in each case arising from the use of plant 85 as the female parent. The cross between plants 90 and 18 also showed a significant difference in the yield of reciprocals but in this instance both parents were moderately self-sterile in selfing tests. However, a high percentage of small plants in the cross 18 × 85 indicated that here again the difference between the yield of reciprocal crosses was due to the contamination of crosses by plants derived from selfing. To reduce the effect of selfing in determining the average yield of single crosses only the member of the reciprocal pair show-

TABLE 2.—THE AVERAGE DEGREE OF CREEPING IN INCHES OF SINGLE CROSSES AMONG SEVEN SELECTED PLANTS OF BROMEGRASS COMPARED TO THOSE OF OPEN-POLLINATION PROGENIES, POLYCROSS PROGENIES, PARENT CLONES AND CHECK VARIETIES (SIX-REPLICATE TEST OF 64 ENTRIES, PLANTED, JUNE 1947, DEGREE OF CREEPING MEASURED, JULY 1948)

Selection	Single cross when selection in column one crossed with:						Average of single crosses	Open-pollination progeny		Polycross progeny	Parent clone
	18	19	83	85	87	90		Source-1	Source-2		
16	17.5	18.0 —	17.5* 17.7	22.2 16.5	15.2 14.5	23.5 21.3	18.9	18.0	19.8	19.7	19.3
18		— 15.0	12.0 11.8	14.3 15.8	15.0 —	15.5 19.0	15.4	15.8	14.0	15.8	12.0
19			15.5 16.7	16.5 —	— 17.2	17.7 —	16.6	13.5	15.7	17.5	15.2
83				13.7 16.3	13.2 13.7	15.0 —	14.6	12.2	13.2	17.0	12.0
85					17.0	18.5 22.5	17.7	17.0	—	15.8	18.5
87						17.3 —	15.8	13.2	16.5	16.2	12.0
90							19.2	19.3	23.7	21.8	21.2
Range							4.6	7.1	—	6.0	9.2
Average							16.9	15.6	—	17.7	15.7
Average all crosses and progenies							16.7				
Checks—Superior							15.2				
Commercial (1)							19.3				
Commercial (2)							19.7				
Parkland							11.2				
Achenbach							19.5				
Av. checks							17.0				
L.S.D. (P = 0.05)—means of 6 plots			3.3								
means of 36 plots			1.3								
means of 6 and 36 plots			2.5								

* The upper value in each cell indicates the degree of creeping of the cross when the selection in column one was the female parent of the cross, i.e. 16 X 83. The lower value in each cell represents the reciprocal cross, or 83 X 16.

ing the higher yield was used for averaging purposes. The member of the reciprocal pair used in calculating average yields was again used in determining the average degree of creeping for single crosses. Only one cross of the group was outstanding for yield. This cross, 90 × 85, showed a yield of 14.3 pounds per plot which is significantly above the yield of 12.7 for the Superior variety from which selections were made.

Table 3 indicates the significance of yield and creeping differences noted in Tables 1 and 2. There were significant differences in yield and degree of creeping among clones, among single crosses, and among open-pollination progenies. For polycross progenies no significance was shown between progenies for yield although there was for the creeping habit. Clones of selections were significantly lower yielding and less strongly creeping than progenies. Single crosses were on the average significantly lower yielding than the other types of progenies. Polycrosses and open-pollination progenies gave similar yields on the average although polycrosses were on the average more strongly creeping than open-pollination progenies. It is interesting to note the absence of a significant interaction between the yield of open-pollination progenies and the source of the seed giving rise to these progenies. On the other hand the significant interaction for the creeping habit indicates that open-pollination progenies derived from Source-1 did not correspond completely with those from Source-2.

The greatest range in yield as indicated in Table 1 occurred within single crosses. The difference between the highest and lowest yielding crosses was 5.3 pounds per plot. The range for the average yield of single crosses was only 1.1 pounds per plot but significance was still shown. Progenies from open-pollination gave a range of 2.2 pounds per plot with differences between strains being significant. For polycross progenies

TABLE 3.—SUBDIVISION OF DEGREES OF FREEDOM AND STRAIN VARIANCE FOR 64 PROGENIES AND CLONES OF BROMEGRASS (YIELDS CORRECTED BY COVARIANCE FOR DEGREE OF CREEPING)

Factor	Degrees of freedom	Mean square	
		Yield	Creeping habit
Checks vs. others	1	11.00*	4.34
Within checks	4	7.21**	84.28**
Clones vs. progenies	1	34.76**	33.03*
Within clones	6	13.57**	92.27**
Single crosses vs. polycrosses and open-pollination progenies	1	112.61**	0.35
Within single crosses	31	10.62**	47.85**
Polycrosses vs. open-pollination progenies	1	3.12	57.30**
Within polycrosses	6	0.24	31.44**
Open-pollination progeny of selection 85 vs. remaining open-pollination progenies	1	1.74	3.98
Between remaining open-pollination progenies	5	5.74**	133.45**
Source of open-pollination progenies	1	0.0	7.00
Source × strain of open-pollination progenies	5	1.40	22.38*
Error—Yield	314	1.71	—
—Creeping habit	315	—	7.96

* Significance P = 0.05.
** Significance P = 0.01.

there was a range of only 0.6 pounds per plot with the differences between selections not exceeding the least significant difference. The range in yield of parent clones of 4.1 pounds per plot was exceeded only by the range for individual crosses. The range in degree of creeping was greatest for individual single crosses (11.7 inches), followed by parent clones (9.2 inches), open-pollination progenies (7.1 inches), polycross progenies (6.0 inches), and the average of single crosses (4.6 inches). The decrease in range of variation of creeping in polycrosses was similar to that shown for yield.

The extent to which the different progeny classes indicated similar combining ability was obtained by making simple correlations between the yields of various progeny classes for each selection. Table 4 presents correlation coefficients obtained from the comparisons of such progeny classes with one another and also comparisons of progeny classes with parent clones. There were no significant correlations between the yields of the different types of progenies although a strong positive relationship was suggested between the yields of open-pollination progenies from the two nursery sources and also between the average yield of single crosses and open-pollination progenies Source-2. Correlations between polycross progenies and single crosses and between polycross progenies and open-pollination progenies were very low. The poor relationship of polycross progeny yields to the yields of other progeny types and the low range of yield among polycross progenies are factors which indicate that the latter may be less suitable than open-pollination progenies for tests of combining ability. Definite conclusions in this regard cannot be made in view of the lack of precision in testing procedure and the limited number of original plants involved in these studies. Much higher correlations were obtained for the creeping habit than for yield. Significant correlations were shown between most of the progeny classes and parent clones. Correlations involving polycross progenies were somewhat lower than correlations involving other progeny classes. It is difficult to determine which progeny class gave the most reliable index of parent character but considering the fairly high relationship between different progenies the degree of creeping shown by any one type may be taken as indicative of breeding characteristics of the parents.

Comparisons of parental clonal lines and their respective progeny classes (Table 4) give indications of the degree to which yield and the degree of creeping were inherited. Correlation coefficients of + 0.21, + 0.35, and + 0.80 were obtained in the comparison of parent yields and the yields of single crosses, open-pollination progenies, and polycross progenies, respectively. Comparisons of the degree of creeping of parent clones and progenies gave correlation coefficients of + 0.97, + 0.87, and + 0.74 for single crosses, open-pollination progenies, and polycross progenies, respectively. It would appear that the spreading character is highly heritable and that rapid changes in the degree of creeping can be accomplished on the basis of selection in parent clonal lines. For yield, however, with a parental range and precision of testing such as encountered in these trials, it would seem that little correspondence may be expected between parent and progeny performance.

TABLE 4.—CORRELATION COEFFICIENTS FOR FORAGE YIELDS AND FOR DEGREES OF CREEPING AS SHOWN BY SINGLE CROSSES, OPEN-POLLINATION PROGENIES, POLYCROSS PROGENIES, AND PARENT CLONES OF SEVEN SELECTED PLANTS OF BROMEGRASS

Comparison	n	r	
		Yield	Creep
Single cross averages and open-pollination progenies Source-1	7	+ 0.44	+ 0.88**
Single cross averages and open-pollination progenies Source-2	6	+ 0.68	+ 0.94**
Single cross averages and polycross progenies	7	− 0.30	+ 0.75*
Open-pollination progenies Source-1 and polycross progenies	7	+ 0.06	+ 0.64
Open-pollination progenies Source-1 and open-pollination progenies Source-2	6	+ 0.73	+ 0.85
Parent clones and single cross averages	7	+ 0.21	+ 0.97**
Parent clones and open-pollination progenies	7	+ 0.35	+ 0.87**
Parent clones and polycross progenies	7	+ 0.80*	+ 0.74

* Significance P = 0.05.
** Significance P = 0.01.

The reliability of single plants as top-cross testers for a group of selections was obtained by a rearrangement of single cross data presented in Tables 1 and 2. Each of the seven bromegrass selections was in turn considered as a top-cross tester for the remaining six plants. When evaluating a plant as a tester all single crosses of it with other plants were extracted and considered as tester crosses. Single crosses of the remaining plants were then averaged to present new values with which tester crosses could be compared. When any plant was treated as a tester, crosses of this plant were not represented in the single cross average as this would have led to exaggerated correlations when tester and single crosses were compared.

As shown by the data in Table 5 there was little relationship between the yield of tester crosses and the average yield of single crosses. This indicates that the plants involved in these studies when used singly in top-crosses cannot be considered reliable testers of combining ability. It signifies in addition that specific combining ability or the particular yield response of a plant in a cross is not closely related to general combining ability or the average behaviour of the plant in crosses. For the creeping habit there was a much better relationship between the response of tester crosses and the average of single crosses and in three of the seven comparisons significant correlations were shown.

The relative importance of specific and general combining ability also was shown by the analysis of variance of single cross yields according to the method of Sprague and Tatum (25). In this method the general combining ability variance results from variation of the mean yields of crosses of parents about the mean yield of the test whereas specific combining ability variance arises from variations of the yields of particular crosses from the yields expected on the basis of average parental performance. As indicated in Table 6 specific combining ability was relatively more important than general combining ability. It would appear that

TABLE 5.—CORRELATION COEFFICIENTS FOR THE YIELD AND THE DEGREE OF CREEPING SHOWN BY TESTER CROSSES AND BY THE AVERAGE RESPONSE OF SINGLE CROSSES FOR SEVEN SELECTED PLANTS OF BROMEGRASS

Tester plant	n	Yield	Creeping habit
16	6	+ 0.27	+ 0.66
18	6	+ 0.28	+ 0.83*
19	6	+ 0.23	+ 0.80
83	6	− 0.73	+ 0.58
85	6	+ 0.56	+ 0.94**
87	6	− 0.42	+ 0.39
90	6	− 0.28	+ 0.82*

* Significance P = 0.05.
** Significance P = 0.01.

TABLE 6.—THE RELATIVE IMPORTANCE OF GENERAL AND SPECIFIC COMBINING ABILITY AS SHOWN BY THE YIELDS OF SINGLE CROSSES OF SEVEN NON-INBRED BROMEGRASS SELECTIONS

Selection	Variance	
	General combining ability	Specific combining ability
16	0.0	0.4760
18	0.1651	0.2032
19	0.0	0.5995
83	0.2395	3.8432
85	0.0	2.0050
87	0.4369	1.7312
90	0.3903	3.7307
Total	1.2318	12.5888

genes with epistatic and dominance effects are more important than genes with additive effects in determining the yields of these bromegrass crosses. Since the degree of crossing was uncertain in some crosses the high specific effects are in part the result of this factor.

The fact that certain selections were partially self-fertile tended to detract from the accuracy of comparisons for yields and creeping determinations but resulted in other observations of interest. The number of small plants was counted in all plots and used as an index of the number of selfed plants in different progeny classes. While classification of plants as selfs on the basis of size is open to serious criticism the method is thought reasonably reliable in this instance in view of the good correspondence of the number of selfed plants indicated by size and the number to be expected from tests of self-fertility of parents plants. Table 7 presents a summary of the percentages of small plants in progenies of selections and the fertility ratings of selections as shown by extensive tests in 1948.

There was a high percentage of small plants in controlled crosses in comparison with the percentages in open-pollination progenies and polycrosses. In addition, the percentages of small plants in all types of progenies bore a rather definite relationship to the self-fertility index of parent

TABLE 7.—PERCENTAGES OF SMALL (LIKELY SELFED) PLANTS APPEARING IN VARIOUS PROGENY CLASSES OF BROMEGRASS AND THE SELF- AND OPEN-FERTILITY RATINGS OF PARENT PLANTS AS SHOWN BY SEED SETTING DETERMINATIONS

Selection	Percentage of small plants				Fertility ratings*	
	Single crosses	Open-pollination progenies		Polycross progenies	Self	Open
		Source-1	Source-2			
16	5.4	1.5	1.5	0.0	2.9	59.2
18	10.0	1.5	1.5	1.5	1.7	9.3
19	1.2	0.0	1.5	0.0	1.1	48.0
83	11.4	3.0	3.0	7.6	12.8	45.3
85	56.4	9.1	—	6.0	22.1	66.6
87	14.4	1.5	0.0	1.5	2.2	45.8
90	1.1	1.5	3.0	6.0	1.1	34.9
Average	14.3	2.6	—	3.2	6.3	44.2

* Expressed in terms of seeds per panicle.

plants. Progenies of plant 85, for example, showed a relatively high percentage of small plants and this plant produced more seed on selfing than did any other plant. The percentages of small plants observed in open-pollination and polycross progenies were much below those expected from the self-fertility ratings of parent plants. Open-pollination progenies and polycross progenies on the average showed 2.6 per cent and 3.2 per cent small plants, respectively while the average set of seed on selfing was 14.2 per cent of the seed set under open-pollination. This suggests the existence of pollen selectivity under free pollination in the field and the possibility of obtaining a reasonably high percentage of crossing between moderately self-fertile plants under natural pollination conditions. Although the number of selected plants in this study was limited there was no indication that highly self-sterile plants gave higher yielding progenies than partially self-fertile plants as found by Tysdal and Kiesselbach (31) for alfalfa. The yields of open-pollination progenies and polycross progenies of plants 83 and 85 were not inferior to the yields of progenies of other less self-fertile selections.

Crested Wheatgrass (Fairway Type)

Non-inbred Material

The analysis of variance of forage yields for the test of non-inbred Fairway material indicated highly significant differences to exist between the 64 strains entering the test. The coefficient of variability for this trial was 17 per cent. Analysis as for a lattice design resulted in an efficiency of 147 per cent as compared to the randomized block design analysis. Average yields were adjusted for block effects and used for all subsequent comparisons of strains. Forage yields of single crosses, other types of progenies, and parent clonal lines are given in Table 8. Since single crosses were not reciprocal they are entered in Table 8, without regard to the direction in which crosses were made. As all plants of this group were highly self-sterile marked differences in the performance of reciprocal crosses were not expected.

TABLE 8.—AVERAGE YIELDS IN POUNDS GREEN WEIGHT PER PLOT OF SINGLE CROSSES, OPEN-POLLINATION PROGENIES, VARIETY TOP-CROSSES AND CLONAL LINES OF NINE SELECTED PLANTS OF FAIRWAY CRESTED WHEATGRASS (SIX-REPLICATE LATTICE TEST OF 64 STRAINS, PLANTED, JUNE 1947, HARVESTED, JULY 1948)

Selection	Single cross when selection in column one crossed with:								Single cross average	Open-poll. progeny	Variety top-cross	Clonal line
	23-7	23-9	23-12	23-14	23-25	30-16	35-4	35-10				
23-5	5.35	6.84	7.02	6.32	5.59	5.56	5.78	6.38	6.10	5.71	6.03	2.12
23-7		6.98	6.12	6.87	5.85	4.59	6.52	6.56	6.10	5.54	5.82	2.20
23-9			6.20	7.10	6.33	6.69	7.10	7.06	6.79	7.43	—	2.03
23-12				7.30	5.78	6.13	6.44	7.35	6.54	6.97	—	3.50
23-14					6.17	5.41	6.66	6.54	6.55	6.35	6.09	2.63
23-25						5.76	5.16	5.82	5.81	5.87	5.52	3.39
30-16							5.51	6.31	5.74	5.89	—	2.93
35-4								6.77	6.24	7.04	—	4.29
35-10									6.60	6.59	6.90	3.22
Average									6.27	6.38	—	2.92
Range									1.05	1.89	—	2.26
Check strains: S-39-$\bar{3}$-1												4.87
S-38-$\bar{9}$-1-3												4.98
S-32-$\overline{28}$-1-1												5.26
S-39-$\bar{7}$-1												4.90
Fairway												5.88

L.S.D.—means of 6 plots 0.95
means of 48 plots 0.34
means of 6 and 48 plots 0.72

Nine single crosses in this test significantly outyielded the Fairway variety. Most of these high-yielding crosses involved plants 23-9 or 23-12 which were selections whose open-pollination progenies also were high in yield. The highest yielding cross, 23-12 × 35-10, gave a yield of 7.35 pounds per plot as compared to a yield of 5.88 pounds per plot for the Fairway variety. The yield of this cross was still below that of the highest yielding open-pollination progeny of plant 23-9 which yielded 7.43 pounds per plot. Most of the low yielding crosses involved plants 23-5, 23-25, or 30-16 which were selections having low yielding open-pollination progenies. The average yield of all open-pollination progenies was slightly but not significantly above the average yield of all single crosses (minimum difference for significance at the 5 per cent level equals 0.25 pounds per plot).

The inter-relationships of yields for various progeny classes and clones are shown in Table 9 by means of correlation coefficients. The significant correlation coefficient of + 0.76 between the average yield of single crosses and the yield of open-pollination progenies indicates that these progeny types gave fairly comparable indices of the combining ability of selections. Correlations were not made between the yields of variety top-crosses and other types of progenies because of inadequate data. However, there appears to have been reasonably good agreement between the yields of top-crosses and open-pollination progenies (Table 8). The small discrepancies between the yields of these two progeny classes were not significant for any of the five selections on the basis of calculated least significant difference values. The non-significant coefficients of − 0.13 between parent clones and single crosses and + 0.32 between parent clones and open-pollination progenies were expected in view of the poor development of parent clones in 1947.

TABLE 9.—CORRELATION COEFFICIENTS OF FORAGE YIELDS OF SINGLE CROSSES, OPEN-POLLINATION PROGENIES, TESTER CROSSES, AND CLONAL LINES OF NINE SELECTED PLANTS OF THE FAIRWAY VARIETY OF CRESTED WHEATGRASS

Comparison	n	r
Single cross averages and open-pollination progenies	9	+ 0.76*
Single cross averages and tester crosses of plant 23-5	8	+ 0.78*
Single cross averages and tester crosses of plant 23-7	8	+ 0.72*
Single cross averages and tester crosses of plant 23-9	8	+ 0.15
Single cross averages and tester crosses of plant 23-12	8	+ 0.38
Single cross averages and tester crosses of plant 23-14	8	+ 0.70*
Single cross averages and tester crosses of plant 23-25	8	+ 0.41
Single cross averages and tester crosses of plant 30-16	8	+ 0.42
Single cross averages and tester crosses of plant 35-4	8	+ 0.86**
Single cross averages and tester crosses of plant 35-10	8	+ 0.72*
Parental clones and single cross averages	9	− 0.13
Parental clones and open-pollination progenies	9	+ 0.32

* Significance P = 0.05.
** Significance P = 0.01.

Each plant was considered as top-cross tester for the remaining plants of the group as has been described for bromegrass selections. When five of the selections were used as tester plants significant correlations were obtained between the yields of tester crosses and the average yield of single crosses. Apparently certain plants may be used for testing the combining ability of selections and be expected to give results comparable to those obtained from crossing selections with several plants. However, when plant 23-9 was used as a tester the yields of tester progenies showed a non-significant correlation of + 0.15 with the average yield of single crosses. This plant is specific in the manner in which it combined with other plants to give vigorous progenies. It is interesting to note that the average yield of single crosses and the yield of the open-pollination progeny of this plant were greater than the yields of comparable progenies of any other selection, that is, general combining ability was also high. Table 10 presents variance values for general and specific combining ability as determined by analysis of single cross yields according to the method of Sprague and Tatum (25). On the whole general and specific combining ability appear equally important in this group of selections since total variance values for each factor are similar. Selections 23-5, 23-14, 23-25, 35-4, and 35-10 show low specific combining ability variances. Except for 23-25 all of these plants when used as individual top-cross testers gave tester yields which correlated well with the average yields of single crosses (Table 9). These plants then would appear to be particularly suitable for testing the general combining ability of new selections in the breeding program.

Inbred Material

In the test of inbred plants of the Fairway type of crested wheatgrass the yields of clonal rows were extremely low and for this reason clonal lines were omitted from the analysis of variance and all subsequent considerations. The analysis of the remaining 42 strains indicated a highly significant difference between progenies for yield of green weight. The coefficient of variability for this test was 17 per cent. In calculating the average

TABLE 10.—THE RELATIVE IMPORTANCE OF GENERAL AND SPECIFIC COMBINING ABILITY FROM THE ANALYSIS OF SINGLE CROSS YIELDS INVOLVING NINE SELECTIONS OF FAIRWAY CRESTED WHEATGRASS

Selection	Variance	
	General combining ability	Specific combining ability
23-5	0.0253	0.0406
23-7	0.0253	0.2339
23-9	0.3297	0.1484
23-12	0.0804	0.2412
23-14	0.0829	0.0644
23-25	0.2735	0.0682
30-16	0.3550	0.2349
35-4	0.0	0.0260
35-10	0.1236	0.0
Total	1.2957	1.0576

yields of single crosses the yields of reciprocals were averaged and the single values obtained used for further averaging. However, in making correlations of the yields of tester crosses, single yields were taken at random from the reciprocal pair to represent the tester cross and to calculate the average single cross yield. This was done to allow the comparison of correlation coefficients on the basis of equal replication. Table 11 presents the yields of all strains included in this test and Table 12 shows correlation coefficients obtained by comparing the yields of different progeny classes and clonal lines.

Single crosses of this group of inbred plants showed a wide range in yield with the highest yielding cross producing 7.16 pounds and the lowest yielding producing 3.01 pounds of green weight per plot. Of the 30 single crosses only one, 41-17 × 44-19, significantly outyielded the Fairway check. On the other hand, nine crosses were significantly lower than the Fairway check in yield of forage. Six of these low yielding crosses included selection 43-15 which was self-fertile to a slight extent. Other low yielding crosses involved selections 41-13 and 43-17. Of the nine crosses present in reciprocal only the 41-17x43-15 cross showed a significant difference between the yield of the reciprocal members. The different yield of reciprocals in this case may be explained by the partial self-fertility of plant 43-15. As in the group of non-inbred plants discussed previously the average yield of all single crosses was slightly below the average yield of all open-pollination progenies but the difference was not significant. (Minimum difference for significance at the 5 per cent level equals 0.31 pounds per plot). The yield of the open-pollination progeny of plant 41-17 is particularly outstanding, being significantly higher than any other open-pollination progeny.

A significant correlation was found between the average yields of single crosses and the yield of open-pollination progenies (Table 12). This suggests that open-pollination progenies may be utilized to obtain a reliable index of combining ability. When individual plants were used as testers a significant correlation between the yields of tester crosses and the average

TABLE 11.—AVERAGE YIELDS IN POUNDS GREEN WEIGHT PER PLOT OF SINGLE CROSSES AND OPEN-POLLINATION PROGENIES OF SEVEN SELECTED INBRED PLANTS OF THE FAIRWAY TYPE OF CRESTED WHEATGRASS (SIX REPLICATE LATTICE TEST OF 49 STRAINS, SEEDLINGS PLANTED, JUNE 1947, HARVESTED, JULY 1948)

Selection	Inbred line	Self-fertility*	Yield of selection in column one when crossed with:						Single cross average	Open pollination progeny
			41–17	43–9	43–15	43–17	44–17	44–19		
41–13	A	0.5	— 5.14	5.05** 5.91	3.76 3.01	4.73 —	— 4.89	4.63 5.12	4.75	5.11
41–17	A	0.0		6.06 6.88	6.70 3.37	6.88 6.61	6.58 6.00	7.16 6.23	6.06	6.68
43–9	B	0.0			5.98 —	5.11 —	5.55 —	6.60 —	5.86	5.49
43–15	C	5.2				3.09 —	— 3.97	3.98 —	4.24	4.84
43–17	C	0.0					4.83 —	4.98 5.81	4.98	5.13
44–17	D	0.0						4.95 —	5.08	5.14
44–19	D	0.0							5.42	5.22
Average									5.20	5.37
Range									1.82	1.84
Check strains—S-39-3-1										4.82
S-38-9-1-3										4.32
S-32-28-1-1										5.45
S-39-7-1										5.20
Fairway										5.91

L.S.D.—means of	
means of 6 plots	1.04
means of 36 plots	0.42
means of 6 and 36 plots	0.80

* In terms of seeds per spike.

** The upper value in each cell indicates the yield of the cross when the selection in column 1 was the female parent i.e. 41-13 × 43-9. The lower value indicates the yield of the cross when the selection in column 1 was used as the male parent i.e. 43-9 × 41-13.

TABLE 12.—CORRELATION COEFFICIENTS FOR FORAGE YIELDS OF SINGLE CROSSES, OPEN-POLLINATION PROGENIES, AND TESTER CROSSES FOR SEVEN INBRED PLANTS OF THE FAIRWAY TYPE OF CRESTED WHEATGRASS

Comparison	n	r
Single cross averages and open-pollination progenies	7	+ 0.81*
Single cross averages and tester crosses of plant 41–13	6	+ 0.83*
Single cross averages and tester crosses of plant 41–17	6	+ 0.26
Single cross averages and tester crosses of plant 43–9	6	+ 0.48
Single cross averages and tester crosses of plant 43–15	6	+ 0.91*
Single cross averages and tester crosses of plant 43–17	6	+ 0.80
Single cross averages and tester crosses of plant 44–17	6	+ 0.93**
Single cross averages and tester crosses of plant 44–19	6	+ 0.98**

* Significance P = 0.05.
** Significance P = 0.01.

yield of single crosses was obtained for four of the seven plants. Selections 41-17 and 43-9 gave results in tester crosses which correlated poorly with the average response of single crosses. This indicates that these selections were selective in the manner in which they combined with other plants. As was observed in the intercrosses of non-inbred plants of the Fairway type those selections showing a high degree of specific combining ability gave high yielding single crosses and open-pollination progenies.

The relative importance of general and specific combining ability variances as shown by single cross yields is given in Table 13. On the whole, the performance of selections in single crosses appears to have been determined more by the general reaction than by specific effect. These results are in agreement with those of Sprague and Tatum in corn (25) where general combining ability was found to be relatively high in inbred lines not selected previously for combining ability. Selections 44-17 and 44-19 appear suitable for testers of general combining ability in view of the low specific variances noted here for their crosses.

TABLE 13.—THE RELATIVE IMPORTANCE OF GENERAL AND SPECIFIC COMBINING ABILITY SHOWN BY SINGLE CROSSES INVOLVING SEVEN INBRED SELECTIONS OF FAIRWAY CRESTED WHEATGRASS

Selection	Variance	
	General combining ability	Specific combining ability
41–13	0.4378	0.4455
41–17	1.9668	0.7255
43–9	0.7245	0.4310
43–15	0.9612	0.7167
43–17	0.1483	0.3692
44–17	0.0	0.0427
44–19	0.0	0.0
Total	4.2386	2.7306

Three single crosses arose from the intercrossing of sister plants of the same inbred lines. An inspection of Table 11 will reveal that the yields of these sib-crosses were not conspicuously lower than the yields of out-crosses of these plants with non-related plants. The yield of the cross 41-17 × 41-13 was intermediate between the average yields of single crosses of the parent plants. The cross 43-15 × 43-17 was low yielding but this may have resulted from the occurrence of selfed plants in the cross as the 43-15 parent was partially self-fertile. The yield of the third cross of related plants, 44-17 × 44-19, was very similar to the average yield of the single crosses of either parent.

Crested Wheatgrass (Standard Type)

The test of seven selections of Standard crested wheatgrass was similar to those for the other species. Ten of the 21 possible single crosses were reciprocal but marked differences in reciprocal performance were not anticipated in view of the high self-sterility of parent plants. The analysis of variance for forage yields indicated a significant difference among the

49 entries involved in the test. The coefficient of variability was 13 per cent. Efficiency when analysed as a lattice test as compared with that when analysed as a randomized block test was 119 per cent. Average yields were corrected for block effects and the corrected yields used for all subsequent comparisons. Table 14 indicates the average yields of all strains included in this test. The method of averaging the yields of single crosses was similar to that described previously for crosses of inbred plants of the Fairway type.

TABLE 14.—AVERAGE YIELDS IN GREEN WEIGHT PER PLOT OF SINGLE CROSSES, OPEN-POLLINATION PROGENIES, AND CLONAL LINES OF SEVEN SELECTED PLANTS OF THE STANDARD TYPE OF CRESTED WHEATGRASS (SIX-REPLICATE LATTICE TEST OF 49 STRAINS, SEEDLINGS PLANTED, JUNE 1947, HARVESTED, JULY 1948)

Selection	Self-fertility*	Single cross when selection in column one crossed with:						Single cross average	Open-pollination progeny	Clonal line
		14-3	14-19	14-20	14-34	19-9	19-16			
14-2	0.1	6.06** 6.37	7.44 —	6.15 6.62	— 6.51	6.02 6.25	— 5.80	6.41	6.15	4.65
14-3	0.2		6.05 7.84	6.90 7.23	— 6.41	6.29 6.60	5.77 6.50	6.54	7.65	6.06
14-19	0.0			— 8.35	6.75 7.68	8.53 —	7.46 7.00	7.62	8.81	4.42
14-20	0.1				7.41 —	8.13 —	— 7.22	7.43	7.23	5.6
14-34	0.0					7.48 7.24	— 5.56	6.74	6.82	4.67
19-9	0.2						— 7.01	7.27	7.76	7.70
19-16	0.6							6.49	6.58	4.22
Average yield								6.93	7.29	5.34
Range in yield								1.21	2.66	3.48
Check strains—S-1333										6.82
Standard (U.S.D.A.)										6.56
S-131										6.41
Forage										7.20

L.S.D. (P = 0.05)—means of 6 plots 0.94
means of 36 plots 0.38
means of 6 and 36 plots 0.71

* Expressed in terms of seeds per spike.

** The upper value in each cell indicates the yield of the cross when the selection in column 1 was used as the female parent, i.e. 14-2 × 14-3. The lower value indicates the yield of the reciprocal cross, 14-3 × 14-2.

A survey of Table 14 indicates good correspondence in the yields of reciprocal crosses. Only in the cross 14-3 × 14-19 was there a significant difference between the yields of reciprocal pairs. As both parents of this cross were highly self-sterile such a difference between reciprocal crosses was not expected. Two crosses, 14-20 × 14-19 and 14-19 × 19-9, were significantly higher in yield than the Forage strain which was the check most representative of the commercial lot from which original selections were made. These crosses both concerned plant 14-19 which on the basis of the average yield of single crosses or open-pollination progeny was the selection giving the highest yielding progenies of all the group. Eight crosses were significantly lower in yield than the Forage check and these crosses in most cases included plants 14-2 or 19-9. These plants, according to the average response of single crosses and open-pollination progenies were plants with low combining ability.

The greatest range in yield occurred among parent clonal lines. The parent clones had a range of 3.48 pounds per plot compared with a range of 2.97 pounds for individual single crosses, 2.66 pounds for open-pollination progenies, and 1.21 pounds for the average yields of single crosses. The average yield of 7.29 pounds per plot for all open-pollination progenies was significantly above the average yield of 6.93 for all single crosses. (Minimum difference for significance at the 5 per cent level equals 0.28 pounds per plot). The lower yield for single crosses conceivably may have arisen due to the presence of small numbers of selfed plants of inferior vigour within them. It is also possible that under open-pollination the original selected plants were subjected to more favourable sources of pollen than available within this relatively restricted group. The average yield of clonal lines was low in comparison with seedling material thus indicating the extent to which clonal material failed to become well-established.

Differential damage to strains for infestations of the western wheat aphis (*Brachycolus tritici* Gill.) was noted in this test and all strains were scored for resistance to damage. A score of 1 represented very severe effects and a score of 10 no injury. The analysis of variance of damage scores indicated a highly significant difference among strains with a coefficient of variability of 14 per cent. Analysis of damage by lattice methods resulted in an efficiency of 196 per cent as compared to the use of randomized block methods. The marked gain in efficiency from lattice methods indicates that infestation in the test area was localized to a high degree. In Table 15 the average resistance to aphis scores for all strains are shown along with self-fertility ratings of the seven parent selections.

Crosses differed greatly in their resistance to the western wheat aphis and in general were less affected than the check Forage strains. Original selections, however, were not selected in this respect. Four of the ten pairs of reciprocal crosses showed significant differences in aphis damage between reciprocals. Parent plants of these crosses were highly self-sterile and differences between reciprocals were not anticipated. It will be observed, however, that in the four crosses where reciprocals differed in reaction the reciprocal member with the lower score arose from the use of the lower reaction parent as female. Apparently selfing was occurring to a higher degree than was anticipated on the basis of tests of self-fertility in parent selections. One of the crosses, 14-34 × 19-9, exhibited almost complete resistance to damage with an average score of 9.88. The most severely damaged cross was 14-2 × 14-3 with a score of 4.61. Both parents of this cross were low in resistance to aphis attack. Open-pollination progenies were significantly less affected than single crosses and

TABLE 15.—REACTION SCORE TO THE WESTERN WHEAT APHIS (*Brachycolus tritici* GILL.) OF SINGLE CROSSES, OPEN-POLLINATION PROGENIES, AND CLONAL LINES OF SEVEN SELECTED PLANTS OF THE STANDARD TYPE OF CRESTED WHEATGRASS. (STRAINS SCORED FROM 1 TO 10 FOR REACTION)

Selection	Self-fertility*	Single cross when selection in column one crossed with:						Single cross average	Open-pollination progeny	Clonal line
		14–3	14–19	14–20	14–34	19–9	19–16			
14–2	0.1	4.61** 5.87	8.05 —	6.42 8.27	— 6.54	7.19 7.98	— 6.31	6.84	7.53	6.85
14–3	0.2		7.65 9.10	7.18 8.55	— 7.84	7.48 7.56	7.99 7.52	7.43	7.96	9.18
14–19	0.0			— 9.25	8.01 8.66	9.38 —	7.97 8.06	8.57	8.97	7.93
14–20	0.1				9.18 —	9.61 —	— 8.98	8.70	9.79	9.79
14–34	0.0					9.88 9.07	— 7.71	8.18	9.21	9.58
19–9	0.2						— 8.05	8.60	8.94	9.98
19–16	0.6							7.80	7.85	9.40
Average resistance								8.02	8.61	8.96
Range in resistance								1.86	2.26	3.13
Check strains—S-1333										8.43
Standard (U.S.D.A.)										8.11
S-131										9.09
Forage										6.97

L.S.D. (P = 0.05)—means of 6 plots	1.14
means of 36 plots	0.46
means of 6 and 36 plots	0.87

* Expressed in terms of seeds per spike.

** Upper value in each cell indicates the resistance of the cross with the selection in column 1 as the female parent, i.e. 14-2 × 14-3. Lower value indicates the resistance of the reciprocal cross, i.e. 14-3 × 14-2.

TABLE 16.—CORRELATION OF FORAGE YIELDS AND APHIS REACTION OF SINGLE CROSSES, OPEN-POLLINATION PROGENIES, TESTER CROSSES, AND CLONAL LINES FOR SEVEN PLANTS OF THE STANDARD TYPE OF CRESTED WHEATGRASS

Comparison	n	r	
		Yield	Aphis reaction
Single cross averages and open-pollination progenies	7	+ 0.77*	+ 0.89**
Single cross averages and tester crosses of plant 14–2	6	+ 0.63	+ 0.91*
Single cross averages and tester crosses of plant 14–3	6	+ 0.51	+ 0.79
Single cross averages and tester crosses of plant 14–19	6	+ 0.74	+ 0.84*
Single cross averages and tester crosses of plant 14–20	6	+ 0.92**	+ 0.95**
Single cross averages and tester crosses of plant 14–34	6	+ 0.83*	+ 0.88*
Single cross averages and tester crosses of plant 19–9	6	+ 0.88*	+ 0.86*
Single cross averages and tester crosses of plant 19–16	6	+ 0.86*	+ 0.76
Parental clones and single cross averages	7	+ 0.28	+ 0.70
Parental clones and open-pollination progenies	7	+ 0.28	+ 0.64

* Significance P = 0.05.

** Significance P = 0.01.

apparently less than clonal lines although the difference was not significant. Check strains varied greatly in aphis reaction with the Forage strain showing more damage than the other three strains.

Correlation coefficients given in Table 16 indicate that there was good agreement between the yields of open-pollination progenies of selections and the average yields of single crosses (r = + 0.77). The yields of tester crosses also correspond well with the average yields of single crosses. Correlation coefficients ranged from + 0.51 to + 0.92 and in four of the seven tester cross comparisons were significant. A non-significant correlation coefficient of + 0.28 was obtained from the comparison of clonal lines and single crosses and also for the comparison of clonal lines and open-pollination progenies. Since the development of clonal material in this test was rather satisfactory these correlation coefficients may be considered reasonably reliable. There was a close relationship also between the aphis reaction for single crosses and open-pollination progenies (r = + 0.89). The response of tester crosses was very similar to the average response of single crosses and in five of the seven comparisons of these two classes of progenies significance was established. Between parent clonal lines and single crosses and between clonal lines and open-pollination progenies there were indications of rather high positive relationships but significance was not shown in either case.

TABLE 17.—THE RELATIVE IMPORTANCE OF GENERAL AND SPECIFIC COMBINING ABILITY FROM SINGLE CROSSES INVOLVING SEVEN STANDARD CRESTED WHEATGRASS PLANTS

Selection	Variance	
	General combining ability	Specific combining ability
14-2	0.3299	0.2376
14-3	0.4120	0.2466
14-19	0.5181	0.2556
14-20	0.4917	0.0
14-34	0.0	0.0742
19-9	0.2086	0.1498
19-16	0.3514	0.0482
Total	2.3117	1.0120

Table 17 gives variance values associated with specific and general combining ability as determined from the yields of single crosses for the seven Standard wheatgrass plants. The total variance for general combining ability is more than double that arising from specific combining ability. This contrasts with the group of non-inbred Fairway selections where the two variances were more or less comparable. Selections 14-20 and 19-16 showed low specific combining ability reactions and would appear most suitable as testers for general combining ability of new selections.

DISCUSSION

Sufficient inter-plant variation in combining ability was exhibited by commercial strains of bromegrass and crested wheatgrass to warrant the general selection of plants in open-pollinated strains on a combining ability basis. Three of the nine plants of the Fairway variety of crested wheatgrass and one of the seven plants of the Standard type were significantly superior to parent strains according to the yields of open-pollination progenies of these plants. None of the seven plants of bromegrass produced open-pollinated progenies which significantly outyielded the parent variety. Significant differences between the yields of progenies were noted, however. Doubtless the exploration of larger populations of these species would reveal a greater range of combining ability among plants and thus provide the possibility of more critical selection.

The relationship of combining ability shown by open-pollination progenies to that exhibited by single crosses was of primary concern in these studies. Interest in this regard arose from the current practice at the Dominion Forage Crops Laboratory of evaluating selections of bromegrass and crested wheatgrass almost entirely on the basis of open-pollinated progeny performance. Controlled single crosses were used as a standard for comparison as this kind of progeny results from a high degree of pollen control except that self-pollination may occur. Polycross progenies also would have formed a desirable standard for the comparison of selections but such progenies were not available for most of the selections tested. The fact that significant correlation coefficients of + 0.76, + 0.81, and + 0.77 were found between the yields of open-pollination progenies and controlled crosses in the three tests of crested wheatgrass appears to justify the practice of selecting crested wheatgrass plants on the basis of open-pollination progeny performance. Lower, non-significant correlation values of + 0.44 and + 0.68 between the yields of open-pollination progenies and controlled crosses in bromegrass were attributed to the inclusion in crossed progenies of selfed plants. Had completedly self-sterile plants been used in the study on bromegrass it is possible that correlations as high as those found in crested wheatgrass would have been obtained. The significant correlations found between open-pollination progenies and those from controlled crosses with regard to the creeping character in bromegrass and the aphis reaction character in Standard crested wheatgrass appear to support the reliability of open-pollination progenies for testing parental breeding characteristics.

Seed for the production of open-pollination progenies can be harvested from selected plants in space-planted nurseries with a very minimum of effort. At this station sufficient seed commonly is obtained from single plants of bromegrass and crested wheatgrass to permit the planting of replicated plot tests or row tests. Where large numbers of selections are to be tested or small amounts of seed are available single unreplicated plots or rows might be sown with frequent checks of standard varieties for comparison. Where comprehensive tests or regional trials are required clonal nurseries of selections would need to be established for selections to produce sufficient seed. Replication and randomization of clones in such nurseries would seem desirable to ensure the pollination of clones with random sources of pollen. In view of the reliability of open-pollination progenies in these studies it would appear that the isolation of such clonal material as recommended by Tysdal and associates (30, 31) for alfalfa is unnecessary and that extensive replication of clones in the clonal nursery may be unwarranted.

Tester crosses made by crossing selections with a single tester plant did not appear wholly satisfactory for testing the general combining ability of selections. In bromegrass the yields of tester crosses of selections did not correlate well with the average yields of more extensive crosses of such selections. Unfortunately the presence of self-fertility in certain bromegrass selections hindered interpretation. In crested wheatgrass approximately half of the plants appeared suitable as testers since the yields of tester crosses involving them were in agreement with the average yields of selections in several single crosses.

Comparisons of the yield variance of single crosses attributable to specific and general combining ability indicated that general combining ability was more important in crested wheatgrass. Specific combining ability appeared relatively more important in bromegrass but the contamination of crosses with selfed plants was responsible for part of this effect. Sprague and Tatum (25), Green (7), and Keller (13) pointed out the specificity of inbred lines and non-inbred plants of corn and noted that the maxium productivity of crosses could be obtained only when the specific reaction was considered. The specific combining ability shown by certain plants of crested wheatgrass is worthy of consideration when composite strains are formed from these plants. Where the composite involves few plants and ultimate production is wanted it would appear necessary that all plants forming the composite perform well in specific combinations with one another.

It was expected that within controlled single crosses certain fortuitous combinations would be found whose yields and plant type would merit an F_1 hybrid program with parents of crosses being increased vegetatively. Certain crosses indicated some hope for such a program. The highest yielding cross of bromegrass yielded 112 per cent of the parent variety Superior. In the Fairway type of crested wheatgrass highest yielding crosses in non-inbred and inbred material yielded 125 per cent and 121 per cent, respectively, of the Fairway check. The highest yielding cross in the Standard type of crested wheatgrass yielded 118 per cent of the Forage check. It is worth noting, however, that the yields of the best open-pollination progenies for these same four groups also were promising being respectively 109, 126, 113, and 122 per cent of check strains for the four groups. This would suggest that initially a hybridization plan might consist of growing single clonal lines in combination with seeded material of current varieties or other strains.

Since inbred and non-inbred materials were available for the Fairway type of crested wheatgrass it was possible to compare in a general manner the frequencies of plants with high combining ability in each group. Critical comparisons were not possible since inbred and non-inbred progenies were tested in different areas of the experimental field and since inbred plants were not related to non-inbred plants. Of the nine non-inbred plants tested the average yields of single crosses for seven were above the yield of the Fairway check with significance being shown in two comparisons. In contrast only one of the seven inbred plants tested gave an average single cross yield above that of the Fairway check with significance not being shown. Tests of combining ability made from 1944 to 1947 in the general crested wheatgrass breeding program at Saskatoon also indicated that plants with superior combining ability occurred as frequently in non-inbred as in inbred strains. The average yield of open-pollination progenies of 102 selfed plants in lines inbred from one to three generations was 98.4 per cent of the Fairway check. The average yield of open-pollination progenies of 31 non-inbred selections was 105.3 per cent of the Fairway check. Of seven plants whose progeny yields were significantly above those of the check, four plants represented inbred lines and three represented non-inbred strains. In view of the high self-sterility of crested wheatgrass and the failure of inbreeding to augment the proportion of selections with improved combining ability it would appear that selfing as a breeding practice to improve the yield of this grass is not particularly promising.

Indications of the heritability of yield and other characters have been provided by correlation analyses between parent clones and progeny classes. A more suitable measure of the inheritance of plant characters is provided by progeny parent regressions with regression coefficients being doubled to provide heritability values (Lush 17). While such a method presumes controlled crossing of selections with common male parentage the technique appears appropriate for polycross and open-pollination progenies where pollination is more or less random. In brome the regression coefficients of the yield of controlled crosses, open-pollination progenies, and polycross progenies on the yield of parent clones were 0.06, 0.17, and 0.11, respectively. Significance ($P = 0.05$) was shown only for the regression of polycross progenies on parent clones. Similar regression coefficients for the degree of creeping of progenies were 0.42, 0.59, and 0.43 for controlled crosses, open-pollination progenies, and polycross progenies, respectively. In this instance all regressions were significant. In the Standard type of crested wheatgrass regression coefficients for progeny yields on parent yields were 0.11 for controlled crosses and 0.20 for open-pollination progenies. Coefficients of 0.51 for controlled crosses and 0.56 for open-pollination progenies were obtained for the regression of aphis reaction of progenies on the aphis reaction of parents. Significance was not shown for any of these regressions. Lower regressions noted for yield than for the creeping character in bromegrass and the insect resistance character in crested wheatgrass indicate the relatively greater need of progeny testing where yield is being studied. Tsiang (29) also noted a lower correlation of selfed lines and clones in bromegrass where comparisons involved yield of forage than where comparisons concerned disease resistance, degree of creeping, and plant height.

The reasonably high regressions of progenies on parent for the degree of creeping in bromegrass is an indication that despite the high-polyploid nature of the species and the irregularity of meiosis as shown by Elliott and

Love (5) there is nevertheless rather stable inheritance for certain characters. Unfortunately, reliable correlations were not available for parent-progeny yield comparisons in Fairway crested wheatgrass; otherwise correlations on diploid, tetraploid, and octoploid levels would have been possible. While the species are different the comparison of the Standard type of crested wheatgrass and bromegrass indicates no marked difference to exist in the general heritability of characters between the tetraploid and octoploid levels.

All comparisons in these studies involved plants growing under space-planted conditions. It is possible that observed differences between strains might have been reduced had plants been growing under conditions of mass seeding. Single crosses containing admixtures of plants arising from selfing likely would have yielded more favourably under mass seeded conditions as a result of the suppression of the smaller selfed plants by crossed plants. The comparative yields of bromegrass and crested wheatgrass under nursery and mass seeded conditions have been studied at this laboratory and moderately good correspondence has been observed. Two composite strains have been formed by grouping clones of the better Fairway and Standard parent selections in these studies. The performance of these composite strains should provide an additional index of the reliability of combining ability procedures which have been recommended.

SUMMARY

1. Studies of combining ability were conducted with single open-pollination plants of commercial strains of bromegrass, Fairway crested wheatgrass, and Standard crested wheatgrass, and for inbred plants of the Fairway type of crested wheatgrass. Combining ability of plants was measured by the forage production of progenies of various types, including controlled single crosses, open-pollination progenies, and in certain cases polycross and top-cross progenies.

2. Significant differences in combining ability were noted among plants of the four groups of plants studied. Three of the nine plants of the Fairway variety of crested wheatgrass and one of the seven of a commercial lot of the Standard type produced open-pollination progenies which out-yielded the parent strain. The combining ability of inbred plants of Fairway crested wheatgrass varied between and within inbred lines but the merit of inbred plants as parents was below that of non-inbred material.

3. Open-pollination progenies of selected plants gave indices of combining ability in agreement with those of controlled crosses in crested wheatgrass but not in bromegrass. Correlation coefficients of the two classes of progenies were $+0.76$, $+0.81$ and $+0.77$ in non-inbred Fairway, inbred Fairway and Standard crested wheatgrass, respectively. In bromegrass non-significant correlation coefficients of $+0.44$ and $+0.68$ were obtained between the yields of controlled crosses and open-pollination progenies derived from duplicate field sources. The lower correlations in bromegrass were considered to result largely from the contamination of controlled cross progenies with selfed plants.

4. Where polycross progenies were available in bromegrass the yields showed a low relationship to the yields of controlled crosses. In the

Fairway type of crested wheatgrass the combining ability ratings for five selections as shown by the yields of variety top-crosses were not significantly different from those shown by the yields of controlled crosses.

5. Single plants appeared quite variable in their reliability as top-cross testers of combining ability. Approximately half of the plants in each of the three groups of crested wheatgrass gave top-cross progeny yields which were significantly correlated with the yields of controlled crosses of these selections. In bromegrass none of the selections was satisfactory as a top-cross tester for other selections.

6. Highly significant correlation coefficients of + 0.88 and + 0.94 were noted in bromegrass between the degree of creeping of open-pollination progenies and the degree of creeping shown by controlled crosses of the same parent plants. In the Standard type of crested wheatgrass a highly significant correlation of + 0.89 was found between the aphis resistance of open-pollination progenies and that shown by single crosses.

7. In both bromegrass and the crested wheatgrasses little relationship was found between the yields of parent clones and their progeny classes. This fact emphasizes the importance of using progeny rather than clonal lines in the evaluation of plants of these grasses for yielding potential. Significant correlations were found between parent clones and progenies in respect to the creeping rooted character in bromegrass and the reaction to aphis character in crested wheatgrass.

8. The results obtained appear to justify the current breeding practice of confining selection to plants of open-pollinated populations or crossed populations and also the evaluation of selections on the basis of open-pollination progeny performance. Since certain plants were highly specific in the manner in which they combined with other selections to give vigorous progenies it would seem necessary to consider the specific response of selections when plants were composited to form new strains.

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A STUDY OF THE HOT WATER TREATMENT OF BARLEY FOR THE CONTROL OF LOOSE SMUT, *USTILAGO NUDA*[1]

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The hot water treatment was first evolved in 1887 by Jensen (13) in Denmark, as a control for smut diseases in general. Since then it has been studied and modified by various workers. Appel (5) used the treatment for the specific purpose of controlling the loose smut of wheat and barley. He emphasized the importance of pre-soaking the seed for a definite period of time at a definite temperature. He believed that pre-soaking the seed broke the dormancy of the mycelium of the parasite so that it could be destroyed more easily by heat. Tapke (16) pointed out the marked influence that cracks in the seed coat have upon damage to the seed from the hot water treatment. He also described methods for applying the treatment on a large scale. Gassner (7) and Gassner and Kirchhoff (8, 9) proposed treating the seed in closed containers. They stated that, under these conditions, intra-molecular respiration took place, with the formation of alcohol and other cleavage products. They considered that these were largely responsible for the control of the disease, and stated that they obtained better results by adding a little alcohol to the water in which the grain was soaked. Dieudonne and Vanderwalle (6) reported that treating barley seed for 20 minutes at 128° F. was ineffective when the seed was not pre-soaked.

It is very important that the seed of promising new varieties, if it carries any loose smut infection, should be freed from this infection before the seed is increased and distributed to farmers. Ordinarily, only the more valuable barley seed is subjected to the hot water treatment, and heavy damage to such seed is a matter of more importance than would be the destruction of an equal amount of ordinary seed. At the commencement of the studies reported below, it was found that the standard schedule, recommended in Canadian Government pamphlets (1, 2) for the application of the hot water treatment to barley seed, destroyed the viability of a considerable portion of the seed, and that the schedule recommended in Leaflet No. 1, Supplement No. 2, of the War Service Committee of the Upper Mississippi Valley Plant Pathologists (3), was even more injurious. Therefore it was decided that the hot water treatment should be investigated under present day conditions in the Prairie Provinces with a view to controlling loose smut with as little injury as is feasible to the seed.

MATERIAL AND METHODS

Barley seed from different places in Saskatchewan, naturally infected with loose smut (*Ustilago nuda* (Jens.) K. & S.), was used in all tests where the plants were raised to the flowering stage and the infection recorded. Spores from representative diseased heads were germinated on agar to make sure that the parasite present was not *U. nigra* Tapke. In germination tests involving only the seedling stage, uninfected seed of varieties commonly grown in Saskatchewan was sometimes used.

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The following method was employed in heating the seed. An electric warming plate with the heating coil under one end gave the required amount of heat for the purpose. The seed was placed in hot water in a glass beaker set inside a slightly larger one, so that the seed did not come in contact with a glass surface resting directly on the hot plate. The beakers could be placed directly over the heating unit or moved away from it, thus securing the temperature desired. While the seed was in the hot water bath, it was stirred with a thermometer. When necessary the temperature of the bath was adjusted by adding hot or cold water.

In larger scale treatments, the seed was placed in baskets made of strap metal frames and copper wire screening, and dipped into water heated with live steam in a concrete tank. The baskets were forced up and down in the hot water bath and the seed was thus spread apart and the water passed through it rapidly so that the seed was all heated at a uniform rate. The temperature of the water was checked constantly with a thermometer and adjusted by passing live steam into the tank.

In those tests designed to show the amount of seed injury as reflected in lower percentage germination, the seed was sown in soil in wooden flats. Three hundred seeds were used for each treatment and this number was sown in each flat. About ten days later, the seedlings were harvested and the percentage that had emerged was computed.

In experiments in which the plants were to be grown to maturity, the seed was sown in soil in one-gallon crocks, for most of the greenhouse tests, and in eight-foot rows for field tests. In the greenhouse tests, four crocks, each sown with about 27 seeds, were devoted to each treatment. In the field tests, about 110 seeds were sown in each row and four or five replications were used. When these tests were harvested, the number of healthy and smutted heads was recorded.

EFFECTS OF VARIATIONS IN THE DIFFERENT STEPS OF THE HOT WATER SCHEDULE ON THE GERMINATION OF BARLEY

The various steps of the hot water treatment schedule were investigated successively to study their separate effects on the germination of the seed. This was done to discover what factors were responsible for most of the reduction in viability.

The first step is the pre-soaking of the seed. In one test three different samples of barley were each divided into six portions, and, after these were pre-soaked at room temperature for periods of time ranging from nil to 25 hours, they were sown without being treated with hot water. It was found that there was no significant difference in the percentage germination of the different portions.

In a second test three samples were each divided into five portions. One portion was neither pre-soaked nor treated and it served as a check. One portion was treated without being pre-soaked, and the remaining portions were pre-soaked for 5, 10, and 20 hours before being treated for 11 minutes at 126° F. (see Figure 1). On the average, treatment without pre-soaking reduced germination by only 3 per cent; but after a 5-hour pre-soak, followed by treatment, it was reduced 20 per cent; after a 10-hour pre-soak, 59 per cent; and after a 20-hour pre-soak, 78 per cent, as compared with the untreated check.

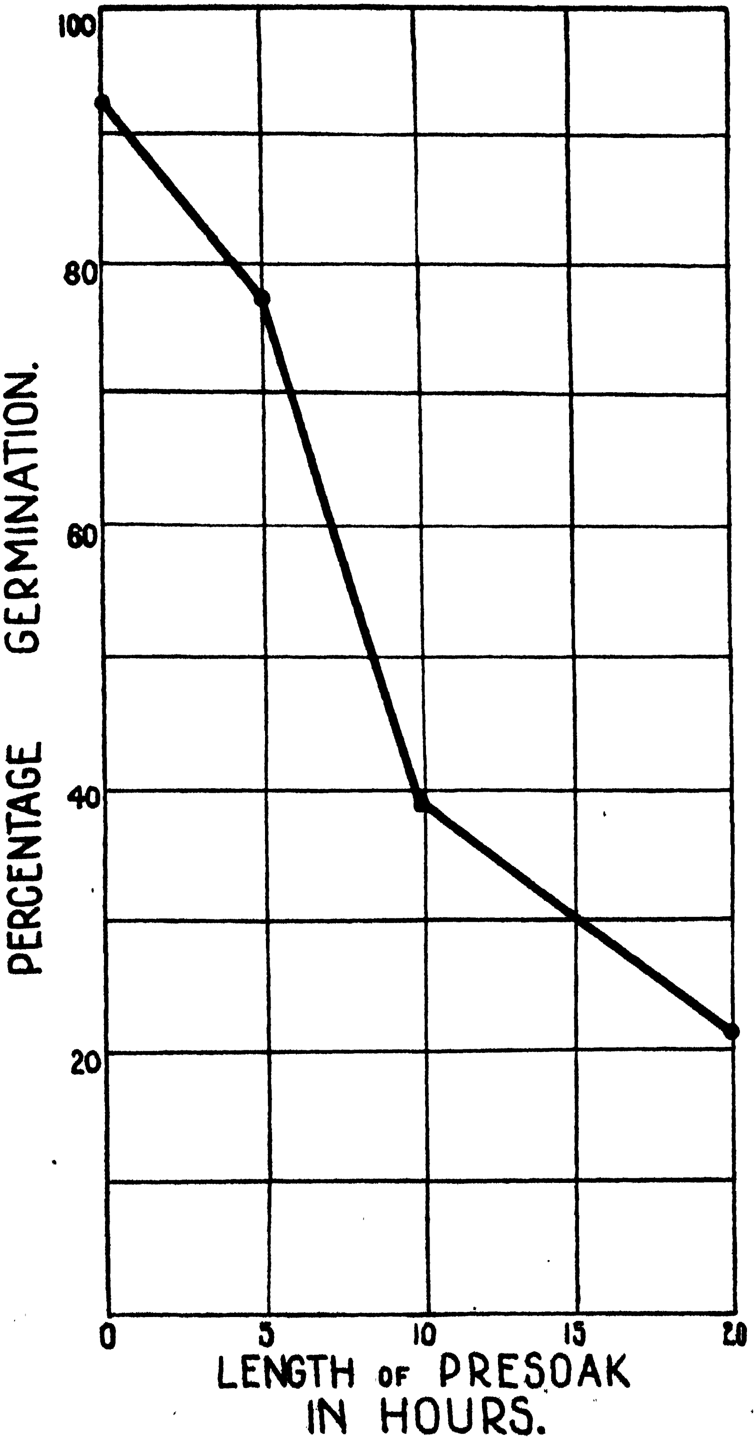

FIGURE 1. The effect of the length of time that the seed is pre-soaked on the germination of treated barley seed.

As a long period of pre-soak caused such severe injury to the seed, it was decided to study the effect of shorter periods of pre-soaking. For a third test, five samples of Montcalm barley were each divided into four portions. Besides the check, which was neither pre-soaked nor treated, one portion was pre-soaked for two hours at room temperature, another for three hours, and a third for four hours, followed by the usual hot water bath. In this test, the average germination of the checks was 95 per cent. When pre-soaked for two hours and treated, the seed had an average germination of 90 per cent; three hours, 86 per cent; and four hours, 79 per cent.

The results of the first test showed that pre-soaking even for relatively long periods, when not followed by the hot water bath, did not depress germination appreciably. In the second test the application of the hot water treatment for 11 minutes at 126° F. had very little effect on the viability of the seed when it was not pre-soaked; but in both the second and third tests, losses in viability increased regularly with each increase in the length of the pre-soak period when these were followed by the hot water bath.

The next step in the hot water treatment is the exposure of the seed to heat in the water bath. Three samples of barley belonging to the following varieties were used: Newal, Plush, and Vantage. Different portions of each sample were pre-soaked for five hours at room temperature and then dipped for 11 minutes in water baths ranging from 120° F. to 130° F. at two-degree intervals (see Figure 2). The average germination of the treated samples varied from 94 per cent, when the treating temperature was 120° F., to 14 per cent, when the temperature was 130° F. The average germination of the untreated checks was 97 per cent. At 126° F., it was 73 per cent and, at 128° F., 48 per cent.

Some reduction in germination took place after treatment at all the temperatures used. The hot water treatment not only reduced the percentage germination, but also retarded germination quite noticeably and tended to weaken the seedlings during the early stages of growth. The rapidity with which the damage to the seed increased at temperatures above 124° F. emphasizes the desirability of treating at the lowest temperature that will insure the control of the disease.

The third step in the hot water treatment, which admits of considerable variation, is the period of time elapsing between treating and sowing. The seed of four different samples of barley was used for this test, and each sample was divided into five lots of 300 kernels immediately after the hot water treatment was applied. In this experiment, all portions of each sample were pre-soaked and treated with hot water in the same manner. After the hot water treatment, the different lots were dried at room temperature for periods of 1, 5, 10, 15, and 20 days, and then sown. There were no marked differences in the average percentage germination of these different lots, although in each instance the lot dried for 5 days germinated slightly better than the ones dried for 1 day, or those dried for 10, 15, or 20 days.

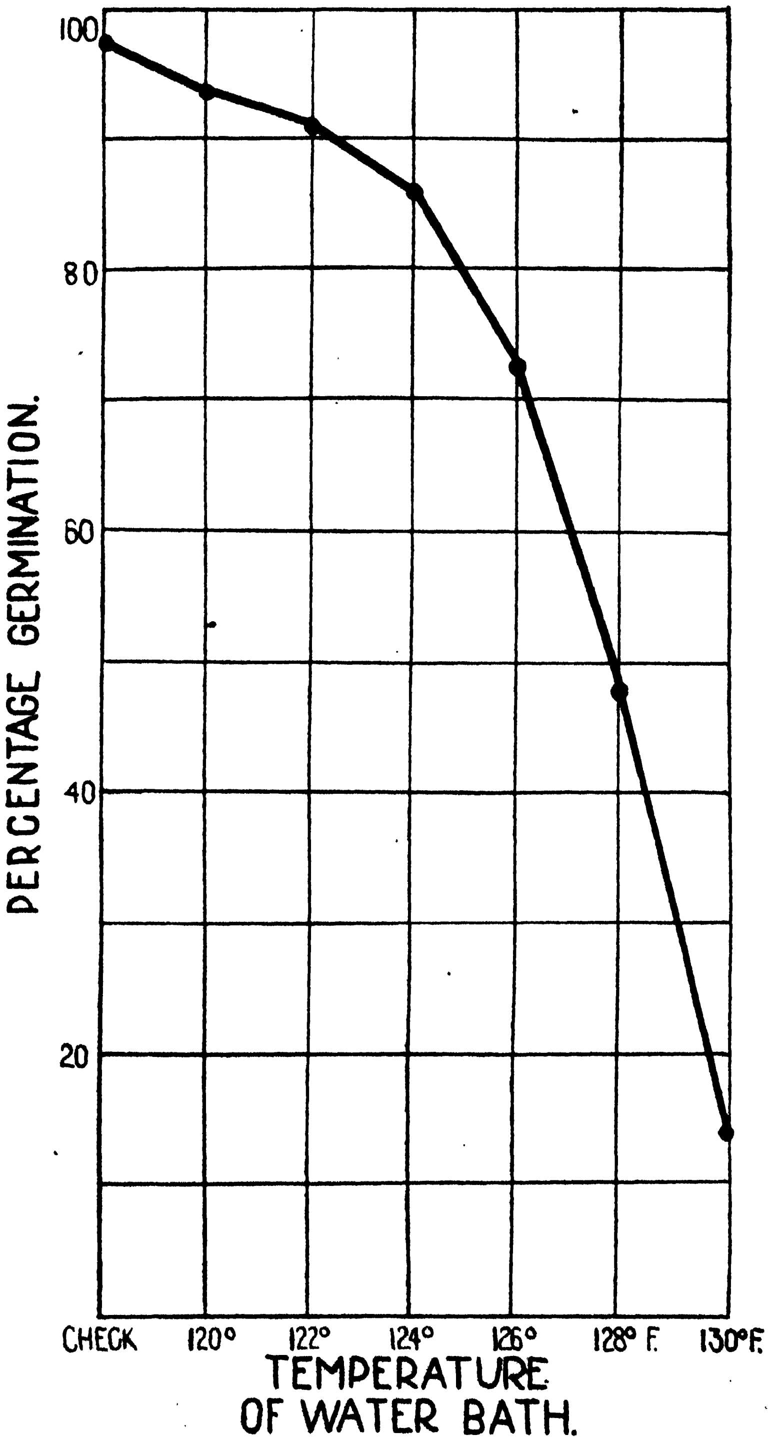

FIGURE 2. The effect of the temperature of the water bath on the germination of treated barley seed.

INFLUENCE OF BROKEN SEED-COATS ON DAMAGE TO THE SEED FROM THE HOT WATER TREATMENT

Besides the studies on the separate effect of each step in the hot water treatment, the effect of mechanical injuries to the barley seed was investigated. Samples of three different varieties of barley were each divided into three portions. One portion was threshed just once, the second was run through the threshing machine three times and the third portion, six times. Then the hot water treatment (pre-soaked 6 hours at 70° F.; dipped 11 minutes at 126° F.) was applied to one-half of each lot, and the other half was sown without treatment.

The effect of repeated threshing was to reduce the percentage germination of the untreated as well as the treated seed. After being threshed once, the untreated seed had an average germination of 89 per cent; after three times, 84 per cent; and, after six times, 52 per cent. Following the hot water treatment, the corresponding figures were 28 per cent, 21 per cent, and 5 per cent. It is evident that damaged seed coats are conducive to greater injury from the hot water treatment. In this experiment, the average reduction in emergence resulting from the hot water treatment of seed threshed once was greater than usual for two reasons: The seed was pre-soaked longer than usual before treatment and one of the samples was particularly sensitive to heat.

GREENHOUSE EXPERIMENTS ON THE CONTROL OF SMUT

Concurrent with the studies reported above, numerous small samples of naturally infected barley seed were treated and sown in glazed earthenware crocks in the greenhouse. The schedule of treatment varied somewhat according to the stage of development of these investigations at the time the different tests were made. Among the sixteen samples of barley tested in this way, at least five of the commonly grown varieties were represented. The average percentage of infected plants in the checks was 8.5. This percentage is not high, but it is representative of the higher infections found in the field in this region. The pre-soak period was usually four or five hours. The temperature of the water bath was 125° F. in most cases, but temperatures of 126°, 127° and 128° F. were used in some of the tests. In only one test out of sixteen did the treatment fail to give complete control of the disease. In this case, the temperature of the water bath was 125° F. As to the effect of the hot water treatment on the yields, the average number of healthy heads in the treated crocks was 54 per cent of the number in the untreated crocks.

One of the samples used in the above-mentioned tests had about 18 per cent of its seeds infected. Its seeds were plump, but they germinated rather poorly, even when untreated. The hulls had been broken off to a considerable extent in the threshing, and the sample suffered severely from the hot water treatment. Two more tests were conducted with it to study its reaction and that of the fungus to milder treatments. The results are summarized in Table 1.

In these tests the pre-soak period was 5 hours. Perfect control was secured by treating the seed for 11 minutes at 122° F. and 124° F. At 120° F., considerable disease developed, and, at 118° F., there was almost

TABLE 1.—EFFECTS OF APPLYING THE HOT WATER TREATMENT AT RELATIVELY LOW TEMPERATURES TO A SAMPLE OF SEED BARLEY

Treatment	Percentage emergence		Percentage diseased	
	First test	Second test	First test	Second test
Untreated check	86.6	89.3	20.6	17.0
Hot water (118° F.)	—	64.2	—	15.6
Hot water (120° F.)	58.3	60.6	3.0	6.2
Hot water (122° F.)	61.4	54.3	0.0	0.0
Hot water (124° F.)	40.0	—	0.0	—

as much smut as in the untreated check. This sample again proved to be very susceptible to heat injury. There was more damage to the seed from treating at 122° F. than ordinarily occurs from treating at 126° F.

After a study of the effect of the pre-soak period on germination had shown that shortening this period reduced the injury to the seed, a test was conducted in the greenhouse to find out how much it could be shortened without interfering with the control of the disease. Three samples of infected barley were used. These were treated for 11 minutes at 126° F., after different portions had been pre-soaked for periods of 2, 3, and 4 hours. About 120 seeds of each variety were subjected to each variation of the treatment. These were sown in a large flat on a greenhouse bench in rows six inches apart. The results of this test are shown in Table 2.

TABLE 2.—INFLUENCE OF THE PRE-SOAK PERIOD ON CONTROL OF SMUT

Sample	Percentage infection in check	Percentage infection after treatment		
		2-hour pre-soak	3-hour pre-soak	4-hour pre-soak
1	7	1.4	0.0	0.0
2	4	2.0	1.0	0.0
3	23	20.0	13.0	4.0

From these results it appears that a longer periof of pre-soak is required for some seed samples than for others. Possibly the condition of the seed coats and rapidity with which water enters the embryo may govern the time required for pre-soaking to ensure the destruction of the parasite within the seed by the hot water bath.

FIELD EXPERIMENTS ON THE CONTROL OF SMUT

A number of tests were conducted over a period of years under field conditions. In these tests, larger populations were used than in the greenhouse tests, but the hot water treatment was applied with the same apparatus as was used in the greenhouse tests. The number of heads in the check rows varied from 500 to 1,500, depending on the variety and the yearly rainfall and other environmental conditions. The pre-soak period was usually 4 or 5 hours. The duration of the hot water bath was 11 minutes. The temperature of the hot water bath varied in different tests and, in some cases, several temperatures were tried with replicate lots of the same sample of seed. All of the seed used bore a natural infection of *U. nuda*. The results of these tests are summarized in Table 3. The percentages of smut are based on the total number of heads, healthy and smutted, but the reductions in yield are based on the number of healthy heads produced in check and treated rows.

TABLE 3.—FIELD TESTS OF THE HOT WATER TREATMENT FOR THE CONTROL OF SMUT

Sample	Percentage of smut and percentage reductions in number of healthy heads										
	Check smut	124° F.		125° F.		126° F.		127° F.		128° F.	
		Smut	Reduction	Smut	Reduction	Smut	Reduction	Smut	Reduction	Smut	Reduction
1	5	0	7	—	—	0	17	—	—	—	—
2	24	—	—	—	—	—	—	0	55	—	—
3	27	—	—	—	—	—	—	0	−23	—	—
4	18	—	—	—	—	—	—	0	73	—	—
5	14	—	—	—	—	—	—	0	33	—	—
6	24	—	—	0	53	—	—	0	69	—	—
7	17	—	—	0	30	—	—	0	53	—	—
8	12	0.1	1	—	—	0.8	14	—	—	0	50
9	11	0	28	—	—	0	47	—	—	0	54
10	11	0	16	—	—	0.05	28	—	—	—	—
11	4	0	22	—	—	0	32	—	—	0	80
12	4	0.2	13	—	—	0	11	—	—	0	54
13	8	0	15	—	—	0	30	—	—	0	63
14	6	0	−5	—	—	0	0	—	—	0	6
15	6	0.2	22	—	—	0	31	—	—	0	28
16	3	0	13	—	—	0	30	—	—	0	74
17	25	0	17	—	—	0	34	—	—	0	76

In the seventeen samples that were tested under field conditions, the average infection of the untreated check rows was 12.9 per cent. As a number of them were not treated at more than one or two temperatures, the average results of the whole series would not give good comparisons. However, nine of the samples were treated at three different temperatures, and direct comparisons can be made with these. At 124° F., the average infection in these nine samples was 0.06 per cent and the average reduction in the number of healthy heads was 14 per cent. At a temperature of 126° F., these two items were 0.09 and 25 per cent, respectively. When the temperature of the water bath was held at 128° F., there was no infection whatsoever but the yield was reduced by 54 per cent.

As a certain amount of infection seemed likely to survive the hot water treatment if the pre-soak period was too short, a field test was conducted on the following plan. Five samples of naturally infected barley were used and portions of each were treated at two different temperatures, namely 126° F. and 127° F., for a period of 11 minutes. Three different periods of pre-soak at a temperature of 70° F. were tried with each. The portions to be treated at 126° F. were pre-soaked for periods of 4, 5, and 6 hours. Those to be treated at 127° F. were pre-soaked for 3, 4, and 5 hours. There were five replications of every treatment in each sample and ten replications of the check. A summary of the results is shown in Table 4.

TABLE 4.—RESULTS OF FIELD EXPERIMENTS TO CONTROL THE LOOSE SMUT OF BARLEY BY MEANS OF THE HOT WATER TREATMENT, APPLIED AT TWO TEMPERATURES AND WITH VARIATIONS IN THE PRE-SOAK PERIOD

Treatment		Number of heads		Percentage infection	Percentage reduction in yield
Pre-soak period	Temperature of water bath	Healthy	Diseased		
Check	—	9344	665	7.1	Nil
4 hours	126° F.	3277	0	0.0	30
5 hours	126° F.	2671	0	0.0	43
6 hours	126° F.	2121	0	0.0	55
3 hours	127° F.	3358	2	0.06	28
4 hours	127° F.	2530	1	0.04	41
5 hours	127° F.	2083	0	0.0	51

The results summarized in Table 4 show that four out of six of the variations of the hot water treatment employed in this test gave perfect control of the smut. However, a small amount of smut appeared after pre-soaking for 3 hours and 4 hours and treating at 127° F. The results of this experiment show that pre-soaking the seed for 5 hours at 70° F. and treating it for 11 minutes at either 126° F. or 127° F. will give perfect control of the disease, but that treating at the higher temperature will result in poorer germination and yield. The reductions in the number of healthy heads show clearly that increasing the length of the pre-soak period or raising the temperature of the main water bath tends to injure the seed and to decrease the yield.

HOT WATER TREATMENT ON A LARGER SCALE

By means of the specially designed baskets, mentioned under "Materials and Methods", several lots of barley seed consisting of from 2 to 5 bushels each were treated at different times over a period of years with quite satisfactory results (15). In 1949, however, five lots of Montcalm barley were treated, and in only one lot was perfect control secured. In all of these larger scale treatments, the seed was pre-soaked at a temperature of about 70° F. and immersed in hot water at 126° F. for 11 minutes. Formerly the seed was pre-soaked for a period of 5 hours, but in 1949 the period was shortened to 4 hours to reduce the amount of injury to the seed. Apparently 4 hours' pre-soak at a temperature of 70° F. was too short, for in four out of five of these samples the average reduction in emergence, resulting from the hot water treatment, was negligible and the control of the smut was not satisfactory.

DISCUSSION

It is obvious, from the results of previous workers and from the experimental data presented in this paper, that satisfactory results from the hot water treatment can be achieved if a proper schedule for its application is followed. However, to secure good control of loose smut without undue injury to the seed, each step in the process must receive proper attention.

Pre-soaking the seed is a very important part of the hot water treatment. The results obtained by Appel (5), Dieudonne and Vanderwalle (6), and Gassner and Kirchhoff (10) stress this point. In one of the tests described in this paper, the author found that the hot water treatment had very little effect on the viability of the seed when pre-soaking was omitted. The data in Table 2 show that pre-soaking the seed makes a great difference to the efficiency of the hot water bath in destroying the fungus.

It is necessary to regulate the temperature at which the seed is pre-soaked as well as to determine the amount of time to be allotted to this operation. According to Güssow and Conners (11), Appel secured complete control by pre-soaking the seed at 86° F. for four hours and incomplete control by pre-soaking it at 64° F. for the same period, followed in each case by the same treatment in the hot water bath. Winkelman (18) specified that the pre-soak should be carried out at a temperature between 76° F. and 87° F. Gassner and Kirchhoff (10) emphasized the marked effect which temperature exerts upon the rapidity with which water is taken in by the seed, the rate of absorption in their tests being 4 times as great at a temperature of 86° F. as it was at 50° F.

The length of time that the seed should be pre-soaked to give best results depends upon several factors, such as the temperature of the water in which the seed is pre-soaked, the condition of the seed coats, and the temperature and the duration of the hot water bath. Gassner and Kirchhoff (10) considered that from 60 to 70 parts of water should be taken up by the embryo to ensure complete control of the disease by a bath of 10 minutes at 125.6° F. Vanderwalle and Larose (17) reported equally good results from pre-soaking for 3.5 hours at 86° F. followed by 10 minutes at 122° F., and as from pre-soaking for 5 hours at 86° F. followed by 15 minutes at 118.4°F. As shown in Table 2, the author found that a 2-hour pre-soak at 70° F. was quite inadequate when followed by a treatment for 11 minutes at 126° F.; a 3-hour pre-soak was somewhat better, but even a 4-hour pre-soak was not sufficient in one case. But in the field test, summarized in Table 4, perfect control was secured by pre-soaking the seed for 5 hours at 70° F. The influence of the length of the pre-soak period upon the viability of the treated seed is strikingly shown in Figure 1.

The temperature of the hot water bath should be chosen to fit in with the pre-soak used and with the length of time the seed is held in the bath. Figure 2 shows the effect of different temperatures on the germination of the seed, and Tables 1 and 3 show the influence of various temperatures on both the seed and the disease. As would be expected, increases in the temperature of the water bath result in increases in seed injury, but the temperature must be sufficiently high to cause some injury to germination or else satisfactory control is not secured. In the present investigations, the reduction in germination usually ranged from 10 to 30 per cent when the disease was completely eliminated. When the seed coats were badly broken by the threshing operation, reduction in germination was sometimes higher. In Saskatchewan, barley is frequently very dry and brittle when threshed and considerable seed-coat breakage results. The treatment of very badly damaged seed with hot water should be avoided.

Different workers have favoured different periods of time for the immersion of the seed in the hot water bath. The standard period recommended in this country has been 10 minutes after a preliminary dip of two minutes in water slightly cooler than the bath. The author believes that a preliminary dip is unnecessary if facilities are present for supplying a relatively large amount of hot water in relation to the quantity of seed placed in the bath and if facilities are available for quickly restoring the proper temperature of the water when the seed is first placed in it. Hence a period of 11 minutes was adopted to make up for the omission of the preliminary dip.

Previous work by various investigators has shown that different samples of grain vary greatly in their susceptibility to injury from the hot water treatment. The results of the present investigations bear out this fact. For instance, the data presented in Table 3 show that, when the seed was treated at 126° F., the reductions in yield varied from nil to 47 per cent. It is felt that one of the main reasons for this phenomenon is differences in the soundness of the seed coats. Tapke (16) showed that the hot water treatment caused more injury to seed wheat when the grain had been threshed by machine than when it had been threshed by hand. The germination tests with seed run through a small thresher, referred to earlier in this paper, showed that the percentage germination of barley seed fell off with increased damage to the hulls and seed coats caused by repeated threshings. On the other hand, it is possible that different varieties of barley differ in their ability to withstand high temperatures in the water bath.

From the results of tests described in this paper, it appears that treated seed, after it is dried, can be stored for a considerable length of time without much reduction in germination when it is sown. The maximum time between treating and sowing in these tests was 35 days, but it is probable that treated seed, if properly dried and stored, could be kept for much longer periods without any marked loss in viability.

There are two hypotheses which may account for the control of *U. nuda* by means of the hot water treatment. The first assumes that the fungus is killed and that the infected embryo of the seed survives the treatment. The second assumes that the embryos carrying the smut are weak and all of them are killed by the treatment, that is to say, if no smut survives. In Table 1 it is shown that treatment at 118° F. reduced germination from 89.3 to 64.2 per cent, a drop of 25.1 or approximately 30 per cent of the emergence of the untreated check. At the same time, the percentage infection was reduced from 17 to 15.6, a drop of 1.4 or about 8 per cent of the infection in the untreated check. This suggests that the infected embryos were not particularly weak, for, of the 25 embryos killed, only 2 were infected.

The following schedule for the application of the hot water treatment to barley seed has given good results during these investigations. Pre-soak the seed in water for five hours at a temperature of about 70° F.; dip the seed for 11 minutes in hot water held at 126° F.; cool immediately in cold water at the end of the 11-minute period; then spread the seed out to dry, and sow it after it has been dried well. It is by no means suggested that this is the only schedule which will give satisfactory results. For example, the seed may be pre-soaked for less time, provided that it is soaked at a higher temperature, as suggested by Lansade (14); or the time and temperature of the hot water bath may be changed. The main point to keep in mind is that the schedule selected should eliminate the smut without causing more damage to the seed than is really necessary.

From the practical standpoint, the use of wire screen baskets is preferable to the use of bags for dipping the seed in the hot water bath. Hanna and Popp (12) did not secure satisfactory results when they treated half-bushel lots in bags, because of the difficulty in heating the seed uniformly.

Several types of machines, such as the Wisconsin hot water treater (4), are now in use in the United States and Canada. By means of these, infected seed can be treated on a comparatively large scale, and they should prove a valuable aid in reducing the amount of loose smut in these two countries.

SUMMARY

1. It is shown that the hot water treatment, applied according to a suitable schedule, will give satisfactory control of the loose smut of barley. However, the treatment generally interferes more or less with the germination of the seed.

2. Raising the temperature or lengthening the time of the pre-soak tends to increase the injury to the seed. Up to a point, each also tends to increase the efficiency of the treatment.

3. Similarly, raising the temperature of the water bath or lengthening the time of treatment causes greater injury to the seed and more thorough destruction of the parasite.

4. The following schedule has been found to give satisfactory results: Pre-soak the seed for five hours at a temperature of 70° F., dip it for 11 minutes in water held at 126° F., cool it immediately with cold water, and dry it well. Other schedules in which the separate steps are properly balanced should give equally good results.

5. It is important that the seed should all become heated to the same degree and at the same rate when it is placed in the hot water bath. For this purpose wire screen baskets are more satisfactory than bags.

6. It is desirable that threshing should be done in such a way as to minimize mechanical injury to barley if the seed is to be treated with hot water.

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VARIATIONS IN VITAMIN CONTENT WITH METHOD OF CURE AND STAGE OF GROWTH OF ALFALFA AND OATS AND THEIR UTILIZATION AS FORAGES BY LAMBS[1]

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The effects of method of cure and stage of growth of plants used as feed for live stock has been the subject of much research. It is now generally accepted that artificial curing of hay is the most efficient method of preservation of vitamins (4, 6, 7, 16) while sun-curing is the most destructive. For most vitamins there is a decrease in their content as the plants mature (3, 9, 17, 19, 24). This indicates the importance of considering these factors when preparing plants as feed for live stock.

Cured forages constitute the major means of satisfying the vitamin A requirements of live stock not on pasture and they also contribute significant amounts of thiamine and riboflavin when incorporated in the rations of non-herbivors. Hence, in view of the established effects of plant maturity and of curing method upon roughage quality, and in view of the supplementary relationships between forages and concentrates in feeding practices, it was deemed worth-while to extend the vitamin assays (23) to include a study of certain forages.

Alfalfa and oats were collected at different stages of growth, cured by several methods, and finally subjected to analysis for thiamine, riboflavin and carotene. The final phase of the study was a feeding experiment in which growth and vitamin A status of blood and liver in feeder lambs were examined.

EXPERIMENTAL PROCEDURE

During the course of this investigation, two series of experiments were undertaken. In 1946, samples of alfalfa and oats were taken at various stages of maturity, cured by different methods, and analysed for thiamine, riboflavin, and carotene. This work was extended in 1948 to include animal feeding trials in a study of carotene content of similar forages and its effect on the vitamin A status of the animals.

First Experiment

The experiment was designed to determine the effects of a number of factors on the vitamin contents of forage crops. Alfalfa (Grimm) and oats (Ajax) were grown on the University Seed Farm in six-row plots replicated in two blocks according to dates of seeding. The plots were harvested at weekly intervals, the first date in each case being the stage just before flowering. Each cutting was divided into three parts of approximately one pound each. The first portion was dried in an oven (11) overnight at 62° C., the second portion in a loft at 18°-23° C. and the third left in the field and turned periodically to expose all parts to the sun. The cured material was ground in a Wiley Mill (sieve openings 1 mm.) and stored in cartons in cold storage at −20° C. Analyses were made for thiamine, riboflavin, and carotene.

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Second Experiment

Forages

This experiment was set up to study carotene values in alfalfa and oats as affecting the vitamin A status of feeder lambs. An attempt was made to repeat the first experiment on a larger scale to include feeding trials. The same varieties of forage were used, and were cut at two stages of growth corresponding to the first and last stages in the first experiment. The methods of cure were also similar. The dried forages were then chopped in a cutting box, thoroughly mixed, bagged and stored in a loft.

Animals

Forty-eight range wether lambs, averaging 65 pounds in weight and 165 days of age, were employed in this experiment. These were divided into twelve groups consisting of two Rambouillets, one Targhee, and one Romnellet per group. The animals were fed low carotene hay until placed in the pens, when they were started on assigned forage rations. The forage was supplemented with a concentrate, adjusted so that each animal received about 12 per cent crude protein. The concentrate for alfalfa forage was made up of beet pulp and oat grain, while that for oat forage contained beet pulp, linseed meal, and oat grain, with ground limestone and iodized salt added in each case. Feedingstuffs analyses of forage and concentrate fed to the lambs were made and total digestible nutrients calculated with assumed digestion coefficients (15).

The lambs were fed individually twice daily and had access to fresh water. Outdoor exercise was allowed for 5 to 6 hours daily, and the animals were bedded with shavings.

Criteria

Data on weights included initial, bi-weekly, and final weights, as well as hot and cold carcass weights. Blood samples, obtained for carotene and vitamin A analyses, were taken at the beginning of the experiment and at the time of slaughtering, and were prepared for analysis according to Kimble (10). Five ml. of plasma from each sample were kept frozen until required for analysis. Bessey *et al.* (2) found no detectable change in vitamin A and carotene content in blood after one month's storage at either 4° C. or − 20° C. Liver samples for vitamin A and carotene determinations, were stored at − 23° C. after quick freezing.

Chemical Analyses

The moisture content of forage was determined by oven-drying at 130° C. for one hour. The crude protein, crude fat, crude fibre, ash, and N.F.E. were determined by the usual feedingstuffs methods. Thiamine was determined by the method of Hennessy (8), modified by Spencer *et al.* (22). It was found necessary for optimal oxidation of thiamine in forage crops to use 5 per cent potassium ferricyanide instead of 1 per cent as used for cereal grains. Riboflavin was determined by the method of Andrews (1), as modified by Spencer *et al.* (22). The method of Nelson (12) was used for determination of carotene, precautions being taken to saturate the column with carotene prior to its assay. For the determination of vitamin A in blood plasma, the method of Kimble (10) was adapted for use with glycerol dichlorohydrin (20, 21). The liver samples were thawed at room temperature, minced, and vitamin A determined according to the method of Gallup and Hoefer (5).

EXPERIMENTAL RESULTS AND DISCUSSION

First Experiment

The values for thiamine, riboflavin and carotene in alfalfa and oats, as affected by stage of growth and method of cure, are summarized in Table 1. Each value represents a mean of duplicate analyses in each replicate, expressed in μgm. per gm. of dry matter.

Considering the effects of methods of curing, these data reveal that the greatest vitamin retention was obtained by oven-drying, and the least by sun-curing. Of the three vitamins studied, carotene was most seriously affected, with a decrease from 482 to 131 μgm./gm.

TABLE 1.—MEAN THIAMINE, RIBOFLAVIN, AND CAROTENE VALUES GROUPED ACCORDING TO DATES OF CUTTING AND METHODS OF CURE

(μgm./gm. Dry Basis)

Method of cure	Thiamine			Riboflavin			Carotene		
Date of cutting	Oven	Sun	Dark	Oven	Sun	Dark	Oven	Sun	Dark
				Alfalfa					
June 4	5.59	4.49	4.44	11.97	9.14	11.38	577	168	447
June 11	4.97	3.74	3.65	11.29	10.56	10.94	564	135	458
June 17	4.08	2.92	3.04	10.21	10.07	9.52	505	140	492
June 22	4.56	3.90	3.46	9.60	9.18	9.62	465	120	410
July 2	3.85	2.98	2.95	9.03	8.60	8.65	401	114	375
July 9	4.75	2.58	2.52	8.77	8.06	6.33	383	111	381
Mean	4.63	3.35	3.26	10.14	9.27	9.74	482	131	427
				Oats					
June 24	3.28	4.60	3.52	7.79	7.12	7.88	331	186	294
July 2	3.40	2.75	3.58	7.02	6.49	6.85	260	93	200
July 12	2.92	2.74	3.28	6.70	5.31	5.91	271	47	199
July 20	3.44	3.61	3.26	5.87	5.53	5.62	234	100	155
July 26	3.98	2.77	3.69	4.77	3.17	3.56	159	80	151
Aug. 2	3.98	3.14	2.96	2.61	3.26	3.20	100	102	114
Mean	3.50	3.27	3.38	5.79	5.15	5.50	226	101	177

TABLE 2.—TOTAL DIGESTIBLE NUTRIENT CONSUMPTION, GAINS AND FEED EFFICIENCY

Forage	Stage	Cure	T.D.N. (average lb.)	Gain (average lb.)	Feed efficiency*
Alfalfa	First	Drier	98.4	19.6	4.89
		Sun	80.3	15.3	5.29
		Loft	87.4	17.0	5.32
	Second	Drier	86.4	19.7	4.44
		Sun	87.7	15.2	5.84
		Loft	92.7	14.0	6.87
Oats	First	Drier	91.9	15.4	6.11
		Sun	90.6	14.0	6.56
		Loft	88.7	11.7	8.70
	Second	Drier	92.2	14.2	6.72
		Sun	87.3	9.7	10.49
		Loft	92.1	12.8	7.47
		Mean	88.4	14.9	6.56

* Feed efficiency value is based on T.D.N. consumed per pound of gain made.

With regard to stages of growth, as shown by dates of cutting, the most significant changes occurred in riboflavin and carotene values. There was a consistent decrease in the values for these two vitamins with advancing maturity.

The trend of thiamine content in relation to maturity of the plant differed in oat and alfalfa forage. Whereas the values in alfalfa gradually declined, there was evidence, in the case of oats, that the corresponding decline of thiamine content in leaves and stems was overshadowed by an increase in the maturing kernel. These data support the findings that oats are higher in thiamine than other cereals, and Robinson *et al.* (14) who showed that as the oat plant ripens the thiamine concentrates in the kernel.

Generally speaking, the vitamin content decreased as alfalfa and oat plants matured. Artificial drying, without exception, resulted in greater vitamin retention, while the most serious losses, especially in carotene, were incurred by curing in the field.

TABLE 3.—GAINS OF LAMBS ADJUSTED FOR T.D.N. CONSUMPTION

Forage	T.D.N. (average lb.)	Gains (average lb.)	Adjusted gains (lb.)
Alfalfa	86.5	16.8	17.3*
Oats	90.5	13.0	12.5
First cutting	88.7	15.5	15.4
Second cutting	88.1	14.3	14.4
Drier-cured	91.0	17.2	16.6**
Sun-cured	86.5	13.6	14.0
Loft-cured	87.7	13.9	14.1
Mean	88.4	14.9	

* L.S.D. at 5 per cent level is 1.9.
** L.S.D. at 5 per cent level is 2.3.

TABLE 4.—DRESSING PERCENTAGE AND CARCASS GRADE

Forage	Stage	Cure	Dressing percentage	Carcass grade*
Alfalfa	First	Drier	46.8	87.8
		Sun	47.4	90.1
		Loft	46.8	91.3
	Second	Drier	48.3	89.0
		Sun	46.2	83.7
		Loft	46.5	82.3
Oats	First	Drier	45.4	80.3
		Sun	44.0	77.4
		Loft	43.9	83.0
	Second	Drier	45.2	80.8
		Sun	43.8	82.5
		Loft	44.6	80.1

* Carcass grade calculated on arbitrary basis of 32 per cent spread between top and bottom carcass.

Second Experiment

A summary of average total digestible nutrient consumption, gains and feed efficiency for each forage is given in Table 2. The feed efficiency values indicate that there was a difference between feeds, alfalfa being superior to oat hay. There was no indication of a difference due to methods of cure or stages of growth. After the gains were adjusted for T.D.N. consumption (18), it was revealed that they were significantly influenced by both the species of forage and the method of curing. Table 3 shows the summary of T.D.N. consumption, gains and adjusted mean gains.

It was found that the dressing percentage based on cold carcass weight was higher for lambs fed alfalfa than for those fed on oat forage. In carcass quality, the animals fed alfalfa were considered somewhat superior. There was no significant difference due to methods of cure or stages of growth. Data relative to carcasses are summarized in Table 4.

The results of the feeding experiment indicate that the carotene content of the forages influenced the vitamin status of the animals, in spite of the fact that the lambs were not initially depleted in vitamin A. No symptoms of vitamin A deficiency were observed during the study. In Table 5 is shown a summary of carotene consumption and vitamin A values in blood plasma and liver.

It was noted that even forages with comparatively low carotene content supplied considerably more than the 50-55 μgm. per kg. per day, suggested as necessary by Peirce (13) for animals recovering from night blindness. There was a gradual increase in vitamin A content in the blood during the experiment regardless of the kind of forage consumed. Only traces of carotene were found in blood plasma and liver.

Vitamin A storage in the liver was influenced by methods of curing as shown by the analysis of variance in Table 6, and which is further illustrated in Tables 7 and 8.

TABLE 5.—CAROTENE CONSUMPTION AND VITAMIN A CONTENT IN BLOOD PLASMA AND LIVER

Forage	Stage	Cure	Carotene consumed (average gm.)	Vitamin A Content Blood Initial (μgm./ml.)	Vitamin A Content Plasma Final (μgm./ml.)	Vitamin A Content Liver (μgm./gm.)
Alfalfa	First	Drier	9.5	0.28	0.32	79.8
		Sun	7.1	0.32	0.64	71.8
		Loft	4.8	0.27	0.35	63.3
	Second	Drier	9.3	0.24	0.36	63.6
		Sun	1.3	0.20	0.27	32.2
		Loft	6.3	0.26	0.27	44.1
Oats	First	Drier	9.6	0.27	0.27	128.3
		Sun	1.9	0.23	0.29	37.4
		Loft	4.9	0.27	0.36	67.4
	Second	Drier	4.4	0.21	0.34	71.5
		Sun	2.3	0.27	0.33	44.5
		Loft	4.3	0.30	0.25	80.8

TABLE 6.—ANALYSIS OF VARIANCE—VITAMIN A CONTENT IN LIVER (μgm./gm.)

Variance due to	D.F.	Mean square
Feeds	1	1893.80
Growth	1	4138.51
Cure	2	6206.55*
F × G	1	497.29
F × C	2	1728.19
G × C	2	1146.29
F × G × C	2	2198.92
Error	36	1089.65
Total	47	

* Significant at 1 per cent level.

While liver-storage of vitamin A was influenced by the level of carotene consumption, the efficiency of conversion of carotene to vitamin A, or the ability of the body to retain it, appears to decrease as consumption of carotene is increased.

It is evident from this study that all forages tested supplied more than the minimum carotene requirements, as indicated by the general increase in vitamin A storage. No forage tested could be considered definitely inferior. Nevertheless, since those feeds high in carotene resulted in the most favourable response, it appears that high carotene content in forages is associated with higher nutritive value in general. It is worth-while to recall that, of the three vitamins studied, carotene was most readily affected by the treatment of the forage.

TABLE 7.—CAROTENE CONSUMPTION AND LIVER STORAGE OF VITAMIN A ARRANGED ACCORDING TO METHODS OF CURE OF FORAGE

Method of cure	Carotene consumption (average gm.)	Vitamin A in whole liver (average gm.)
Drier	8.2	42.8
Loft	5.0	30.3
Sun	3.2	22.4

TABLE 8.—CORRELATION BETWEEN CAROTENE CONSUMPTION AND LIVER STORAGE OF VITAMIN A

Source	D.F.	Correlation coefficient
Between crops, stages and interactions	3	
Between cures and interactions	8	0.8537*
Error	36	−0.0036

* Highly significant.

SUMMARY

Two investigations of the effects of stage of maturity and method of curing of alfalfa and oat forage are discussed.

In the first, thiamine, riboflavin and carotene content of the cured forages were determined and it was found that oven-drying (62° C.) effected the greatest vitamin preservation and field-curing the least, with loft-curing being intermediate. Values obtained at six stages of maturity indicated consistent decreases in vitamin contents with the exception of thiamine in oats. Carotene was most readily affected by curing and stage of maturity.

In the second, the forages were similarly cured but represented early and late stages only. The feeds thus obtained were individually fed over a 70-day period to fattening lambs receiving a concentrate allowance in addition. The gains of the lambs fed artificially-dried forages were greater than those receiving sun or loft-cured products. Forage treatments did not influence carcass quality although, as with gains, alfalfa-fed lambs were superior. Plasma vitamin A values did not reflect forage treatments but there was a significant positive correlation between carotene intake and liver vitamin A at slaughter.

While it is true that no forage tested was subjected to excessive weathering during curing, it is significant that those samples higher in carotene produced better responses in the feeding trial and, of the vitamins studied, carotene was most readily destroyed by adverse treatment of the forage.

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SOIL MICROFLORA IN RELATION TO MANGANESE DEFICIENCY[1]

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It has been demonstrated by Gerretsen (4, 5) and later, using Gerretsen's technique, by Leeper and Swaby (6) that manganese in the soil can be oxidized by the soil microflora. More recently, however, Bromfield and Skerman (2) reported that the organisms isolated on calcium citrate-manganous sulphate medium (5) were able to oxidize manganese only in the presence of citrate but not in the unsterilized soil-agar plaque. Therefore they concluded that the oxidation of manganese observed on a citrate medium is apparently due to a non-biological oxidation. The organisms, owing to their activity, increased the alkalinity of the medium and at this level of pH manganese is readily oxidized in the presence of oxyacids.

Continuing an investigation on the relationship of soil microflora to the manganese deficiency in soil (8, 9), several organisms capable of oxidizing manganous salts on artificial media as well as in soil agar plaques have been isolated on calcium citrate-manganous sulphate agar (5).

The organisms isolated from these soils belong to several groups of bacteria, proactinomycetes, actionomycetes and fungi. The following fungi proved to be able to oxidize manganese on calcium citrate-manganous sulphate medium as well as in the soil-agar plaques: *Helminthosporium victoriae*, *Culvularia lunata*, *C. brachyspora*, *C. inaequalis*, *Periconia circinata*, *Cephalosporium* sp. and several as yet unidentified cultures. These organisms also were able to oxidize manganese in media containing only manganous salts of manganese, agar and tap-water. The data presented in Table 1 indicate that these fungi were capable of oxidizing manganese in these simple media. However, a culture of bacterium was able to oxidize manganese only from the citrate salt. On inoculation of these fungi into soil-agar plaques (Gerretsen's modified technique) the organisms were capable of oxidizing $MnSO_4 . H_2O$ in sterilized as well as in unsterilized soil. From Figure 1, it is quite apparent that various nitrogenous compounds added to the soil prior to plating (to give equivalent weights of N) influenced the intensity of manganese oxidation. Thus, addition of asparagine stimulated the process of oxidation, whereas addition of $Ca(NO_3)_2$ retarded it. Apparently different sources of nitrogen affected the metabolic processes of the organisms differently.

In this respect Funchess (3) and later Ridalevskaya (7) demonstrated that soils with strong nitrifying power or on the application of nitrates tended to increase the water-soluble fraction of manganese in the treated soils. Furthermore, it has been shown by Bokuchava (1) that nitrates are able to inhibit the activity of catalase. Taking these findings into consideration, it is possible to assume that the application of different nitrogenous fertilizers may affect not only the rate of their growth (Figure 1) but also the activity of their metabolic by-products as well.

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[1] Contribution No. 304 (Journal Series) from Division of Bacteriology and Dairy Research, Science Service, Canada Department of Agriculture, Ottawa.

[2] Bacteriologist.

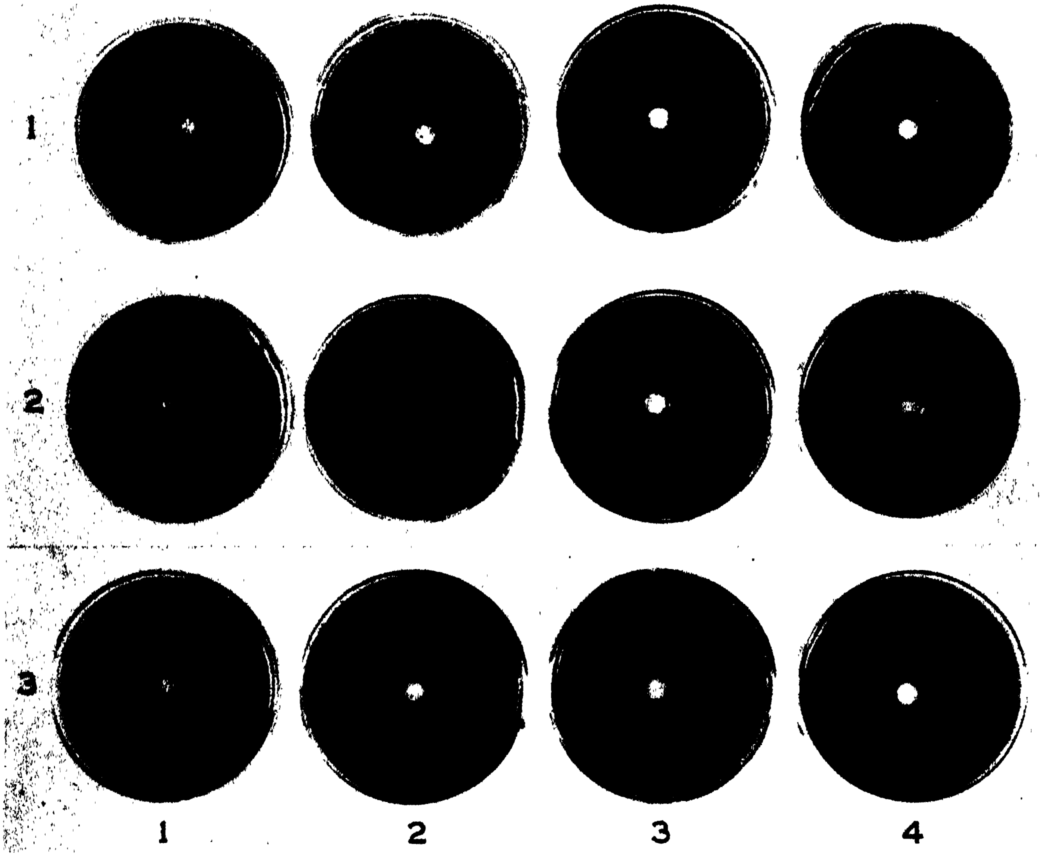

FIGURE 1. Influence of Different Sources of N on the Rate of Oxidation of $MnSO_4$ (incubation—6 days at 23° C.).

Horizontal rows 1—Control soil
2—Asparagine added,
3—$Ca(NO_3)_2$ added.

Vertical rows 1—Sterilized, inoculated,
2—Unsterilized, inoculated,
3—Sterilized, not inoculated,
4—Unsterilized, not inoculated.

The results herewith presented indicate that it is possible by means of Gerretsen's method to isolate fungi capable of oxidizing manganese without the presence of citrate in artificial media. Furthermore, when these organisms were introduced into a soil-agar plaque they were able to oxidize manganous sulphate in sterilized as well as in unsterilized soil.

TABLE 1.—OXIDATION OF DIFFERENT MANGANOUS SALTS BY SOIL MICRO-ORGANISMS

Organisms	$MnCO_3$	$MnCl_2$	$MnSO_4$	Mn. Citrate
C. brackyspora	+ + + +	+ +	+ + +	+ + + +
C. lunata S	+ +	+ + +	+ + + +	+ + + +
C. inaequalis	0	+ +	+ + +	+ + + +
P. circinata	0	+ + + +	±	+ + + +
N-4-2 (F)	+ + + +	+ + + −	+ + +	+ + + +
Bact. N-4-Mn	0	0	0	+ +

Rate of oxidation:
+ + + + Very good
+ + + Good,
+ + Fair,
+ Poor,
0 No oxidation.

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THE CLIMATE, SOILS, AND SOIL-PLANT RELATIONSHIPS OF AN AREA IN SOUTHWESTERN SASKATCHEWAN[1]

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INTRODUCTION

Differences in plant life between localities have probably been evident to man since his creation. These changes in the vegetation are usually associated with latitude, longitude and elevation. Within soil zones the changes are due to soil differences, topography and exposure.

In this paper, certain of the relationships that exist between plant communities and the soil, and climatic factors that influence their growth, are studied. The area lies within the mid-grass prairie formation in the southern part of the province of Saskatchewan, Canada.

REVIEW OF LITERATURE

Soil-plant relationships have long been recognized by the majority of workers in the fields of ecology, geobotany and pedology. Weaver (19) states "The fundamental relation in plant ecology is that reciprocal one between the plant and its habitat."

In many studies of soil-plant relationships, the effects of climate have not been assessed fully. However, to obtain a proper perspective of the habitat, the interdependency of soil, plant growth and climate must be considered and weighed, so that each contribution can be assessed according to its share.

Moss (9) mentions the striking correlations between the vegetation and the soil texture, being especially marked in sandy loam and other light soils. He also states that there is a close relationship between prairie vegetation and topography. Even the small variations of micro-relief were often found to be accompanied by pronounced differences in grass flora. Colvin and Eisenmenger (5) recognized two schools of thought regarding the major factors controlling plant distribution. One theory expounded contends that the chemical properties of the soil are the factors controlling plant distribution (Salisbury, 11), and (Silva, 16). The second supposition is that the physical properties of the soil are of primary importance in determining the presence or absence of a particular species (Shantz, 14). Bennett (1) states soil type exerts marked influence on vegetation characteristics, because of the various adaptive factors involved. Hicock (7) found no correlation between soils and vegetation. He attributes this to the fact that climate in some instances may compensate for the poverty of certain soil conditions.

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Tansley (17) has stated the problem where he mentions the difficulty in explaining the features of different kinds of soil, and of their relations to vegetation. He states: "Pedology is developing very rapidly and we are still largely ignorant of the exact ways in which different soils affect plants". Investigation of these questions leads straight to some of the most difficult problems of plant physiology and physical chemistry.

METHOD OF PROCEDURE

Existing soil survey maps (Mitchell *et al.*, 8), and information from previous surveys in connection with land use studies were used as a base for this study. Fifty sites supporting native vegetation were selected. These represented the five different soil textual classes in the area, namely, fine sandy loam, light loam, loam, clay loam and clay. All sites were located within a radius of fifty miles from Swift Current, Saskatchewan. As far as possible the sites selected were not overgrazed and in many cases a near climatic climax condition was observed. After the study had progressed sufficiently to warrant elimination, sixteen of the sites were discarded, as they were found unsuitable because of heterogeneous soil conditions and overgrazing. In many cases, the vegetal covers on the remaining sites had been changed in past years by grazing, mowing, burning, drainage and soil drifting, but the effects of these factors were indiscernible at the time the study was made.

Composite soil samples were collected from all sites. These were analysed for moisture equivalent, which gave a comparative figure for moisture retentivity of the soil. Wilting coefficient was determined by the indirect method based on that outlined by Work and Lewis (21). Determination of soil texture was made using the hydrometer method as outlined by Bouyoucos (2).

The vegetation was sampled by means of meter quadrats, and the point method as outlined by Clarke *et al.* (3).

NATURAL RESOURCES OF THE AREA

The area selected for the study lies within the Brown soil zone. This is very similar to the Brown soils of the United States. The Saskatchewan Haverhill association corresponds closely with the Scobey area of the United States. It occupies the short grass prairie (*Bouteloua-Stipa*) and the adjacent section of the mixed grass prairie (*Stipa-Bouteloua-Agropyron*) of Saskatchewan and Alberta at elevations of 1800 to 3800 feet. This is the most arid section of the province and is characterized by grassland vegetation, the absence of trees and the frequency of drought. The average precipitation ranges over different parts of this region from eleven to fifteen inches. The Brown soils have been developed over glacial moraine deposits which are essentially extremely variable in grade composition and topographic expression.

The surface area is undulating to rolling, broken by a number of valleys, escarpments, plateaux, and isolated hills. Other important physical features of the area include inter-moraine drainage basins of lakes Chaplin and Johnston, and the large areas of dune sand found in the Great Sand Hills west of Swift Current, Saskatchewan.

Although a large portion of the drainage is local, there is considerable spring run-off into the Saskatchewan River system through the Frenchman River along the southern section of the region. Local drainage is carried by shallow coulees and creeks into small lakes or sloughs, which are shallow and often saline. There is seldom any flow except during the spring run-off period.

The area under study has been covered by at least three advances of the Keewatin ice-sheet. Consequently, all soils are derived from the material deposited by the retreating and melting glaciers. Post-glacial climate has affected the soil development that followed glaciation. It has produced a soil that ranges in colour from light to greyish brown. The "A" horizon is up to six inches in depth, and covers a layer of calcium carbonate accumulation. The vegetational cover is a grassland climax, the dominants being both short and mid-growing species.

CLIMATE

Precipitation is the principal climatic factor that limits the growth of vegetation in the Brown soil zone. The area studied has a typical semi-arid climate and is situated in the centre of a broad ecotone with the mid-grass prairie to the east and north and the short-grass prairie to the west and south. As Swift Current, Saskatchewan, is located in approximately the middle of the study area, meteorological data obtained from the Dominion Experimental Station, Swift Current, were used. It was felt that this data would be comparable for the area used in this study. For a 28-year period, the total annual precipitation has amounted to 13.28 inches, with 7.04 inches falling during the growing season (Table 1). The annual precipitation, which fluctuates from year to year, shows no definite cycles of wet or dry. However, the tendency is for wet years to be very wet and dry years to be moderately dry.

Evaporation from a free water surface is 29.32 inches for the five summer months, giving a precipitation-evaporation ratio of 0.45. This high evaporation makes the rainfall relatively inefficient. According to Sanderson (12) the area studied has a semi-arid climate and an annual water deficiency of 8 to 10 inches. This water deficiency is large and imposes serious limitations on agriculture.

The snow, which accounts for 22.6 per cent of the annual precipitation, has an average depth of 30.1 inches. Winter thaws caused by chinook winds, which often melt much of the snow, occur occasionally, but lakes, sloughs and creeks remain frozen throughout the winter.

The area is characterized by a very wide range in temperatures between the extremes of summer and winter. The mean annual temperature is 38.4° F., the highest recorded temperature being 103° F. in June and July, and the lowest −55° F. in February. The winters are long and rigorous. The prevailing winds, which are from the southwest, are usually warmer than those from other directions, making the study area warmer and the temperature more variable than in the eastern and northern sections of the province. There are approximately 120 days between the last killing frost (29° F.) of the spring and the first killing frost of the fall.

TABLE 1.—METEOROLOGICAL DATA—DOMINION EXPERIMENTAL STATION, SWIFT CURRENT, SASK., 1922-1949 (28 YEARS)

Month	Temperature—° F.			Precipitation			Bright sun	Wind velocity	Evaporation
	Mean maximum	Mean minimum	Mean	Rain	Snow	Total precipitation			
				(inches)	(inches)	(inches)	(hours)	(hourly)	(inches)
January	18.9	0.2	9.5	0.04	5.8	0.62	93.8	7.4	—
February	21.7	2.8	12.3	0.00	4.7	0.47	122.1	7.7	—
March	33.5	15.3	24.4	0.05	4.9	0.54	169.8	8.1	—
April	52.2	29.0	40.6	0.58	2.3	0.81	205.4	8.5	—
May	64.6	39.0	51.8	1.53	0.4	1.57	236.6	8.7	5.54
June	70.8	47.3	59.0	2.81	0.0	2.81	227.6	7.5	5.79
July	80.4	52.4	66.4	1.85	0.0	1.85	301.8	6.2	7.43
August	77.4	49.1	63.2	1.74	0.0	1.74	256.6	6.2	6.41
September	65.9	39.8	52.8	1.03	0.7	1.10	194.7	6.7	4.15
October	54.2	29.7	41.9	0.53	1.8	0.71	159.6	7.3	—
November	35.0	16.1	25.5	0.07	4.7	0.54	96.7	7.5	—
December	23.0	4.8	13.9	0.04	4.8	0.52	79.7	7.6	—

In spring the native vegetation begins growth as soon as temperature conditions are favourable and maintains a rapid rate of growth until moisture supplies are exhausted, at which time the native grasses begin to dry and give the prairie a characteristic brown colour. Dry, hot winds in the summer have a marked desiccating effect on the soil and vegetation.

These climatic factors exert a definite influence upon soil development and native vegetation. Those factors of greatest importance are the relatively low precipitation, high evaporation, and extremes of temperature.

SOILS

The Brown soil zone comprises approximately 20,000,000 acres in Saskatchewan. It includes all of the shortgrass prairie formation, as well as parts of the mid-grass prairie formation in the eastern and northeastern sections of the zone. The soils are brown in colour as the result of the small amount of organic matter that is produced under the relatively low rainfall of the area. The lime layer, or horizon of calcium carbonate accumulation, is generally encountered eight to twenty inches below the surface.

The parent material of the soils studied is varied, but all of glacial origin. A large portion of the area has developed from undifferentiated boulder-clay deposits. This includes Haverhill light loam, loam, and clay loam. Others, including the most productive, have developed from clay deposits occupying the beds of former glacial lakes, namely, Sceptre heavy clay. The Hatton fine sandy loam has developed on sandy, glacial lake and alluvial deposits. The climate, and indirectly the soil, are probably the most important factors in determining the type of vegetation that will exist on a given soil. However, it has been observed that *Haplopappus armerioides* and *Mentzelia decapetala* occur apparently only in areas where the parent material is Bearpaw shale.

The light soils are usually well drained, the topography being classed as undulating to gently rolling. Stones are rare but gravelly sub-soils are common. In consequence of the sandy nature of the soil, the moisture-holding capacity is low. However, on the more level areas, the water table often is relatively high, permitting deep-rooted plants such as hoary sage *Artemisia cana* Pursh, to grow abundantly. Moisture penetration is also deeper in the lighter soils. Saline sloughs are common, the dominant salts being sulphate of calcium, magnesium and sodium. The lighter soils are characterized by a sparse population of the more drought-resistant plant species. Also, there is greater evidence of wind erosion. Figure 1 illustrates typical sandy soil topography.

The topography of the medium textured soils is gently rolling (Figure 2), and the vegetation of these soils is usually devoid of *Artemisia cana*. Drainage is good with the exception of a few shallow depressions. Stones are few to common, with more occurring in the steeper rolling phases. These soils have developed largely from undifferentiated glacial till.

The clay soils of the area are characterized by an almost flat topography and extremely few stones. Surface drainage is adequate but profile drainage is relatively slow owing to the high clay content of the soil.

The presence of an accumulated lime layer ($CaCO_3$) is characteristic of the soils under a grassland climax in areas of limited rainfall. This accumulation generally takes place in the B horizon. The C horizon, which is composed of the parent material, is little affected by the soil-forming processes. Climate and vegetation, which are the most important of these processes, act only on the A and B horizons.

Some studies have been made in an effort to establish a definite relationship between the chemical composition of native plants and that of the soils on which they grow (Scott, 13), (Daniel and Harper, 6).

Table 2 shows the percentage of three important minerals found in the soil of the area studied. Until further work is done on the available nutrients in the soil and the actual requirements of plants, it is difficult to to state whether or not minerals are a limiting factor in the growth of native plants. At Manyberries, Alberta, it was found that barnyard manure spread over native pasture at the rate of fifteen tons to the acre increased the yield of grass, in some cases as much as four times. However, commercial fertilizers showed no beneficial effect. In most cases, water is such a dominating factor in controlling plant growth that it masks all others.

TABLE 2.—PER CENT TOTAL NITROGEN, PHOSPHORUS AND POTASSIUM ON UNCULTIVATED SURFACE SOILS

Soil texture	Per cent of nitrogen	Per cent of phosphorus	Per cent of potassium
Hatton fine sandy loam	0.17	0.05	1.48
Hatton light loam	0.20	0.04	1.70
Haverhill loam	0.20	0.06	1.60
Haverhill clay loam	0.23	0.05	1.69
Sceptre clay	0.24	0.08	1.76

Saskatchewan Soil Survey Report No. 12. (Mitchell *et al.*, 8).

VEGETATION

The vegetational cover of the area studied is a grassland climax. Scattered shrubs and small trees may grow in the vicinity of more favourable areas such as natural depressions, sloughs, and streams.

The area studied is within the mixed grass prairie as shown by the map (Figure 3). Perhaps a better name is the mid-grass prairie, as it is a mixture of the short and mid-grasses. The dominant grasses of the true tallgrass prairie do not appear in this association.

Weaver and Clements (20) considered the area to be a grassland climax with the mixed grass prairie, *Stipa-Bouteloua*, as an association and shortgrass, *Buchloe-Bouteloua*, a grazing disclimax rather than a true association as named earlier by Clements (4). Shantz (15) recognized the shortgrass formation, and the tallgrass formation which contained numerous associations, but he made no mention of the mixed grass prairie. It is the writer's contention that there are three grassland formations of the Great Plains prairie area of Canada, namely, the shortgrass (Figure 4), the mid-grass (Figure 5), and the tallgrass. In the shortgrass formation of Saskatchewan, the following associations are found: *Bouteloua-Carex*, *Bouteloua-Stipa*, *Bouteloua-Agropyron*, and an *Agropyron* consociation. From evidence

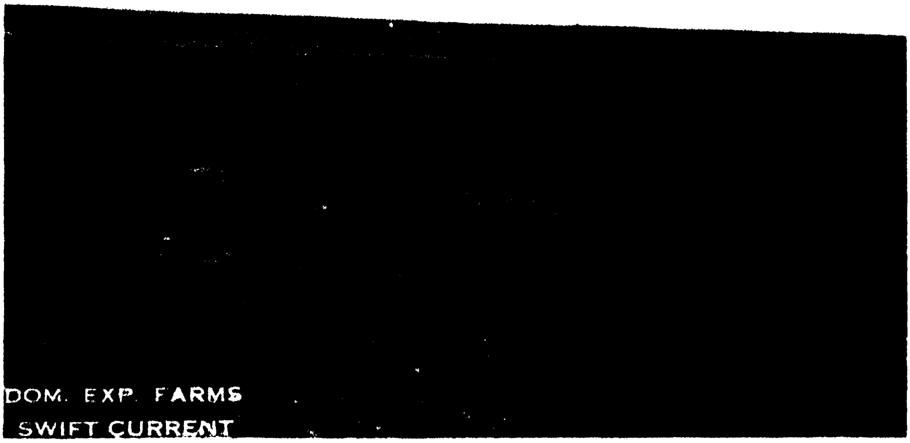

FIGURE 1. Typical sandy soil topography. This area has been heavily grazed. Notice the exposed clumps of *Opuntia polyacantha* Haw.

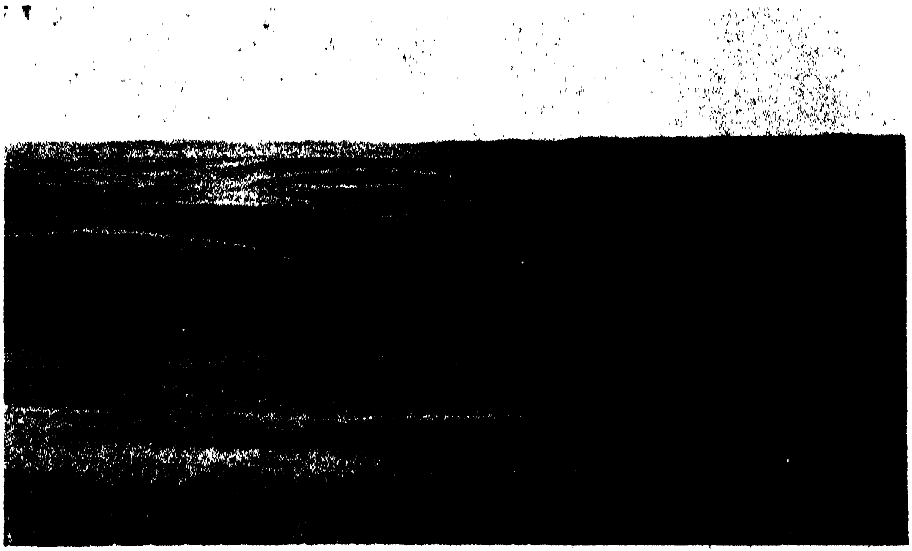

FIGURE 2. A loam soil illustrating the topography which is rolling, being a succession of knolls and depressions.

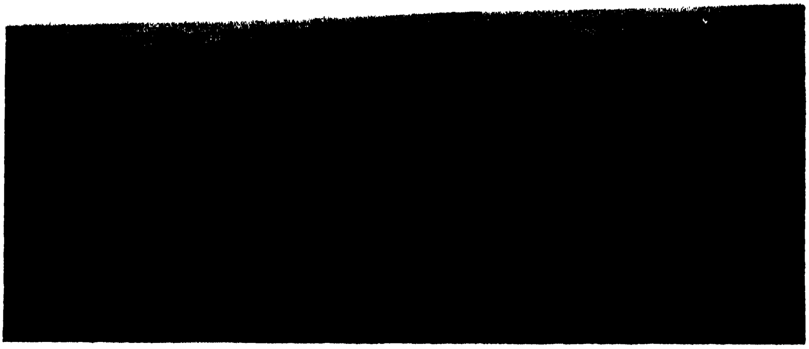

FIGURE 4. Good quality Herefords grazing on typical shortgrass prairie. The dominants are *Bouteloua gracilis* and *Stipa comata*.

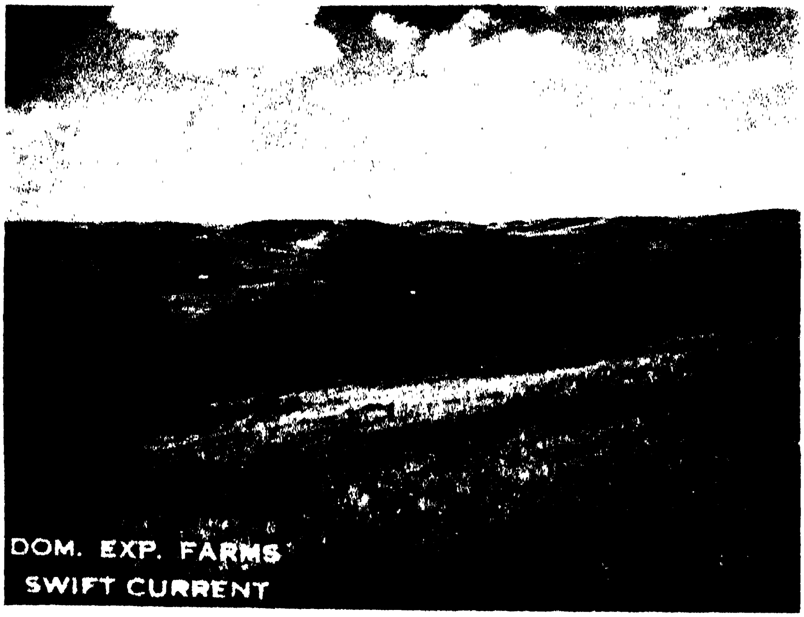

FIGURE 5. Typical mixed grass prairie. *Stipa comata*, *Agropyron Smithii*, and *Bouteloua gracilis* are the principal species.

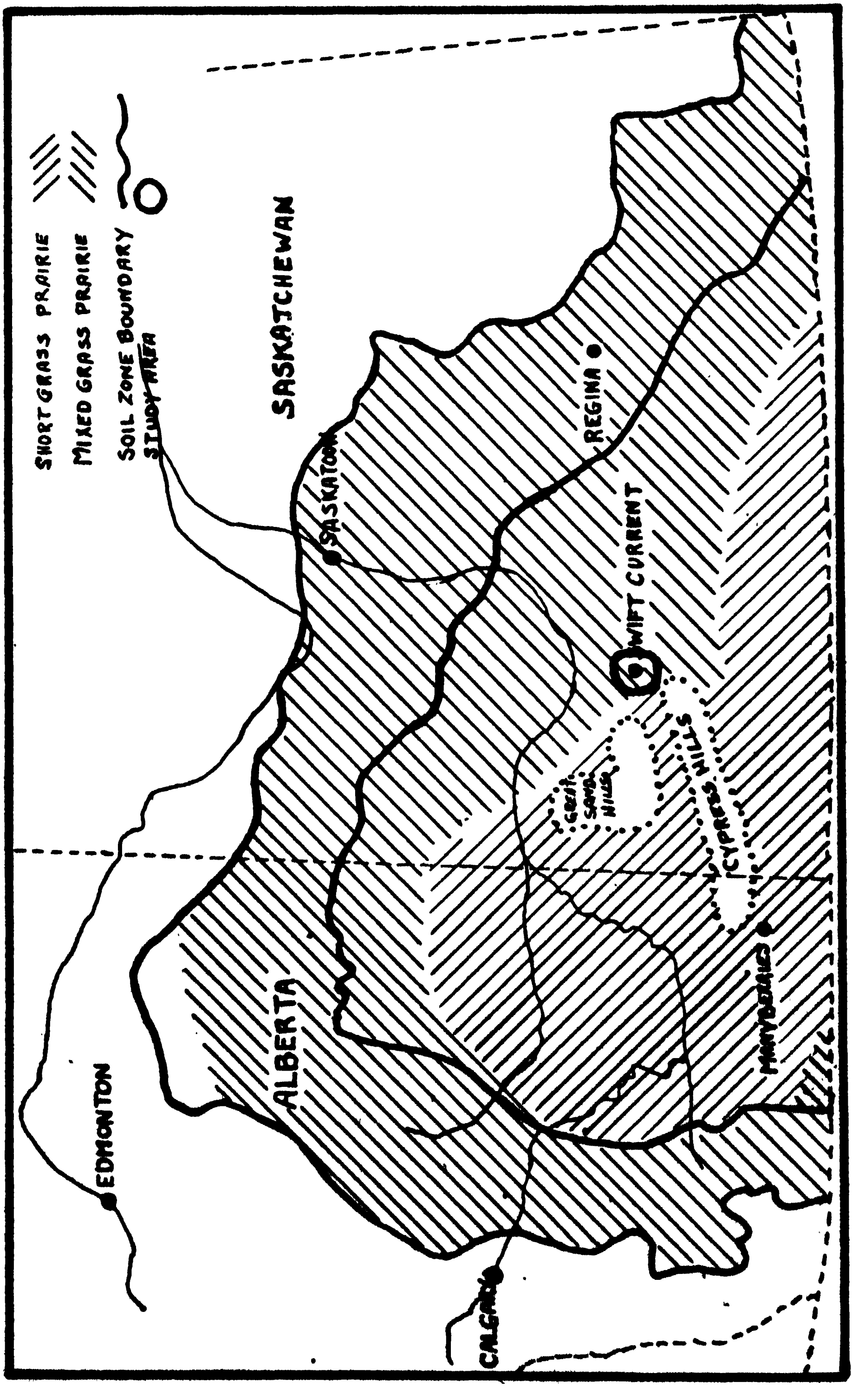

FIGURE 3. The location of the mid-grass prairie. Notice the boundaries of the area known as the shortgrass prairie.

exhibited in exclosures, which have been protected for eighteen years at Manyberries, Alberta, it would appear that, for southern Saskatchewan at least, a true shortgrass association exists rather than a disclimax caused by grazing. In the mid-grass formation of which the study area is part, there are several associations, namely, *Stipa-Bouteloua*, *Bouteloua-Stipa*, *Agropyron-Stipa viridula* and an *Agropyron* consociation.

In the mid-grasss formation the two main associations are *Stipa-Bouteloua* and *Bouteloua-Stipa*. These are very closely related. The balance between these two associations is so close that very little biotic or climatic disturbance is needed to change the cover from one to the other. In many areas a change may take place similar to that suggested by Weaver and Clements (20). That is, a climax stand of *Stipa-Bouteloua* or *Bouteloua-Stipa*, after a few years of overgrazing, may become a grazing disclimax dominated by *Carex eleocharis* Bailey, and *Bouteloua gracilis* (H.B.K.) Lag. In the mid-grass prairie area, the *Bouteloua-Stipa* association will not revert to a *Stipa-Bouteloua* association because of shallow soil together with the low annual rainfall and high evaporation rate. Consequently, it appears to be a true association. Under heavy grazing in the *Bouteloua-Stipa* association, the *Stipa* almost disappears and the *Bouteloua* may decrease or increase with a marked increase of *Carex eleocharis*.

The shortgrass association of the mixed-grass formation is characterized by two dominant grasses, *Bouteloua gracilis* which occupies over one-third of the total vegetative cover, and the speargrass *Stipa comata* Trin. and Rupr., which forms an integral part of the *Stipa-Bouteloua* association. Other commonly associated grasses are *Agropyron Smithii* Rydb., *Koeleria cristata* (L.) Pers., and *Poa secunda* Presl., which occupy varying percentages of the third total grass cover and are listed in their usual order of dominance. Grasses of lesser importance are *Calamagrostis montanensis* Scribn. and *Muhlenbergia cuspidata* (Torr.) Rydb. The most common sedge is *Carex eleocharis* Bailey, while *Carex filifolia* Nutt., is of frequent occurrence. Associated species are *Artemisia frigida* Willd., *Phlox Hoodii* Richards, *Malvastrum coccineum* (Pursh) A. Gray, *Pulsatilla ludoviciana* (Nutt.) Heller, *Androsace septentrionalis* L. var. *puberulenta* Rydb., *Gutierrezia diversifolia* Greene, and *Chrysopsis villosa* (Pursh) Nutt. Other species of less importance include *Atriplex Nuttallii* S. Wats. and *Opuntia polyacantha* Haw. *Selaginella densa* Rydb. occupies a large area, but exerts very little influence on the association. Of the shrubs, *Artemisia cana* is the most important with *Rosa Macounii* Greene playing a minor role except in sandy areas.

The short grasses are well adapted to the climatic conditions and light soils on which they exist. *Bouteloua gracilis* forms a very dense sod, competing keenly for all available moisture, and practically excluding all other grasss where moisture is in short supply.

The mid-grass association of the mixed grass formation is dominated by *Stipa comata* and *Bouteloua gracilis*. The growth conditions supporting this association are much more favourable than those conditions within the area dominated by the shortgrass association.

The mid-grass association is made up of two layers of vegetation. Such species as *Bouteloua gracilis* and *Carex eleocharis* form the lower layer beneath *Stipa coma'a*, *Agropyron Smithii*, and *Koeleria cristata*. In a climax stand the competition between the two layers of vegetation seems to have reached a state suited to both. The short grasses possess the advantage of suffering less from evaporation and, because of their low growing nature, they are well able to withstand grazing. It is doubtful if lack of light is any disadvantage to the shorter growing plants in this area.

Associated grasses of the mid-grass association are *Stipa spartea* var. *curtiseta* Hitchc., *Agropyron trachycaulum* (Link.) Hitchc. var. *unilaterale* (Cassidy) Malte., *Agropyron dasystachym* (Hook.) Scribn., *Stipa viridula* Trin. and *Festuca scabrella* Torr. The latter may be found only in favoured places. The principal sedge is *Carex eleocharis*, while *Carex filifolia* and *Carex heliophila* may be found locally. In the lower areas, where moisture conditions are favourable to mesic species, *Carex atherodes* Spreng., and *Carex rostrata* Schw., may be found.

The shrubs found in this association are *Rosa Macounii*, *Symphoricarpos occidentalis* Hook., *Elaeagnus commutata* Bernh., and *Artemisia cana*. *Salix* spp. and *Populus tremuloides* Michx., occur under better than average moisture conditions, such as adjacent to sloughs, streams, and rivers.

The mid-grass association (*Stipa-Bouteloua*) is characterized by a large number of associated species which may be rare or abundant, depending on conditions. A few of the more important ones are: *Artemisia frigida*, *Phlox Hoodii*, *Malvastum coccineum*, *Pulsatilla ludoviciana*, *Liatris punctata* Hook., *Haplopappus spinulosus* (Pursh.) D.C., *Selaginella densa*, *Solidago* spp., *Gutierrezia diversifolia*, *Glycyrrhiza lepidota* (Nutt.) Pursh., *Eurotia lanata*, *Atriplex Nuttallii*, *Thermopsis rhombifolia* (Nutt.) Richards, *Astragalus pectinatus* (Wood) Dougl., *Chrysopsis villosa*, *Lydodesmia juncea* (Pursh.) D. Don., and *Androsace septentrionalis* var. *puberulenta*.

The principal life forms are the shortgrasses and the mid-grasses. These exist on all the sites studied but in various proportions of the total vegetational cover. The perennial forbs exist throughout the area with a larger proportion in evidence on the loam soils and a comparatively few on heavy clay. *Artemisia frigida* is the most common forb and can be found over the entire area. In many cases it serves as an indicator of overgrazing.

Productivity of the grasslands increases from the lighter soils to the heavier soils. The density of the grass cover shows the reverse trend (Table 3). The explanation is due to the difference in forage composition, palatability of the species, and their growth habits. For example, *Bouteloua gracilis*, which makes up over fifty per cent of the total grass cover on fine sandy loam, is very palatable, but its low-growing, mat-forming habit makes little of it available for live stock, with the possible exception of sheep.

To illustrate further, a 100 per cent density stand of *Stipa comata*, which is used as a standard for establishing yields (*Clarke et al*, 3) has been calculated to produce 5000 pounds per acre, while a 100 per cent density stand of *Bouteloua gracilis* yields only 1600 pounds per acre, or 0.31 *Stipa* equivalents. Consequently, the lighter soils which have a high percentage of *Bouteloua gracilis* yield less because the dominant grass. *Bouteloua gracilis* only yields approximately one-third of that of *Stipa comata*, which is the dominant on the medium textured soils, and only one-quarter as much as *Agropyron Smithii* which dominates the heavier soils.

TABLE 3.—THE YIELD AND PER CENT COVER OF GRASSES AND SEDGES, AS DETERMINED BY THE POINT METHOD, FOR FIVE SOIL TEXTURE CLASSES

Soil texture	Density of grass cover in per cent	Estimated yield in lb. per acre using point yield method
Fine sandy loam	13.16	302*
Light loam	13.27	329
Loam	11.52	448
Clay loam	11.19	371
Heavy clay	6.91	452

* Method used in determining yield described by Clarke *et al.* (3).

RESULTS

Vegetation has been used to indicate land suitable for agricultural purposes, ground water, and recently, as a geophysical instrument (Rankama, 10), to detect the presence of certain ores. This has been possible by studying the soil-plant-climate relationships as they exist. It is obvious that vegetation, if correctly interpreted, will answer many questions regarding the soil and climatic conditions of the region.

In a study of this kind dealing with soil-plant relationships, climate undoubtedly confuses the issue. The interaction of vegetation and climate has greatly modified the earth's surface, so that now we have many soils formed from various parent materials differing in colour, texture and structure. This has led to a close correlation between the soil and the vegetation which it supports. However, for a small area such as that used for the study, no correlation appeared to exist between the parent material of the soil and the vegetation.

To simplify the problem, the climatic data as presented earlier are assumed to be the same over the area studied. Making this assumption, the problem then becomes a soil-plant relationship study. However, soil moisture must be considered, although it is an indirect factor dependent on the soil texture which determines the moisture-holding capacity of any particular soil. Moisture may be concentrated in low areas due to topography, but as these areas only confuse the problem they were avoided. Very little is known at present of the water requirements of native plants or their physiology. Until more is known it is almost impossible to say whether a plant grows on a certain area because of local moisture conditions, or because of soil texture, or whether it is a combination of both. Because climatic data could not be obtained for each site they have been omitted as a variable with the knowledge that the omission may be the source of considerable error. Soil under this condition then becomes the most important factor controlling plant growth. The major differences in soils are caused primarily by the method of deposition, the nature of the parent material, and soil evolution. These differences are evident in the results of mechanical analysis, moisture equivalent, and wilting coefficient tests. Table 4 summarizes the data. From Table 4 it is seen that the lightest soil, fine sandy loam, contains a high percentage of sand and a relatively low amount of clay particles. Likewise, the moisture equivalent and the wilt-

TABLE 4.—PROPERTIES OF VIRGIN SOILS IN THE BROWN SOIL ZONE OF SASKATCHEWAN EXPRESSED IN PER CENT

Soil	Moisture equivalent		Wilting coefficient		Mechanical analysis					
	0–6 inches		0–6 inches		0–6 inches					
					Range			Average		
	Range	Average	Range	Average	Sand	Silt	Clay	Sand	Silt	Clay
Fine sandy loam	7.19 to 18.54	11.25	3.27 to 6.50	4.40	49.39 80.37	11.41 40.29	7.81 11.42	67.22	23.09	9.83
Light loam	18.15 to 28.94	20.59	6.42 to 10.31	8.31	53.25 77.15	14.96 29.08	7.89 18.76	58.69	20.89	19.61
Loam	17.12 to 24.41	21.06	6.35 to 10.87	8.05	40.81 47.01	35.90 41.35	14.58 19.60	43.81	38.08	18.77
Clay	21.55 to 35.45	27.04	7.84 to 15.27	10.12	20.40 44.13	29.44 50.70	22.86 48.40	35.38	37.33	27.34
Heavy clay	36.03 to 46.88	42.66	17.55 to 20.15	19.39	11.98 15.72	16.60 24.12	60.26 68.20	14.61	21.42	63.98

ing coefficients are low. As the clay content increases and the percentage of sand decreases, the soils are able to hold more moisture as indicated by the increase in the moisture equivalent and the wilting coefficient.

Clay contains a large percentage of particles of colloidal dimensions which naturally exhibit the properties of colloidal systems. One of these properties is the retention of water by imbibition, whereas silt and sand particles hold water only on their surfaces.

Theoretically, a clay soil should be able to support a more mesic type of vegetation than any of the soils studied. In sand there exist many mesic plants, almost all of which are deep-rooted, while shallow plants are unable to obtain moisture because of the rapid percolation of precipitation.

Because of the colloidal properties of the clay particles in heavy clay soils, many grasses seem unable to live. The swelling and shrinking of the clay particles causes the soil to crack. This cracking is very characteristic of heavy clay soils and appears to be detrimental to the root systems of bunch-grasses. In the case of *Agropyron Smithii*, which is rhizomatous, the tearing action only serves to separate young plants from the mother plant rather than destroying the crown and exposing many of the roots, as is apparently the case with *Stipa comata* and *Bouteloua gracilis*. However, there may be a competitive factor related to soil moisture that is at present not recognized.

In Table 5 the dominant grasses for the heavy clay soils are shown to be *Agropyron Smithii* and *Stipa viridula*. If grazing takes place the decrease in *Stipa viridula* is very marked. The dominant forbs of heavy clay soils are *Phlox Hoodii*, *Artemisia frigida*, and *Eurotia lanata*, in this order.

Table 6 shows clearly the close positive correlation between *Agropyron Smithii* and the clay content of the soil. From Table 5 it is evident that *Stipa viridula* is also correlated with clay content. *Bouteloua gracilis* tends to decrease as clay content increases (Table 5). Consequently, on the sandy soils, *Bouteloua gracilis* occupies over a third of the total grass cover, and in some instances as much as ninety per cent. *Stipa comata* and *Koeleria cristata* are present on all soil textures and show no apparent correlation with the percentage of clay present. From visual observation, this is true of *Koeleria cristata*. However, *Stipa comata* is generally the dominant grass on loam soils, but it is usually absent from the heavy clays. Often biotic or climatic factors make *Bouteloua gracilis* co-dominant on the lighter soils but *stipa comata* is often the dominant grass on newly stabilized dune sand areas. In this study pure sand has been disregarded because of its unstabilized nature. Other grasses of minor importance are *Stipa spartea* var. *curtiseta* which appears to reach its maximum development on loam soils; *Calamagrostis montanensis* which is equally distributed over the various soil types with the exception of heavy clay; *Muhlenbergia cuspidata* which usually indicates eroded slopes and hilltops; *Calomovilfa longifolia* (Hook.) Scribn. which is almost exclusively a sand plant; and *Poa secunda* and *Avena Hookerii*.

Of the forbs, *Artemisia cana* is the only one in this area that might be used as a soil indicator. While it occurs on all soil textures, it reaches its maximum development on light, well drained soils. *Selaginella densa*

TABLE 5.—CORRELATION OF NATIVE FLORA TO SOIL TEXTURE IN THE BROWN SOIL ZONE
SPECIES EXPRESSED IN PER CENT DENSITY OF TOTAL GROUND COVER

	Fine sandy loam	Light loam	Loam	Clay loam	Heavy clay
Grasses and sedges:					
Bouteloua gracilis	9.50	7.51	3.48	3.55	0.00
Stipa comata	2.02	2.22	3.49	3.01	0.00
Koeleria cristata	0.20	0.31	0.86	0.63	0.62
Agropyron Smithii	0.34	0.86	1.01	0.94	4.55
Stipa spartea var. curtiseta	0.05	0.18	0.72	0.42	0.07
Poa secunda	0.04	0.06	0.23	0.46	0.00
Muhlenbergia cuspidata	0.00	0.00	0.03	0.00	0.00
Calamagrostis montanensis	0.01	0.02	0.04	0.01	0.00
Stipa viridula	0.00	0.00	0.00	0.09	1.95
Avena Hookerii	0.00	0.00	0.00	0.01	0.00
Calamovilfa longifolia	0.04	0.00	0.00	0.00	0.00
Carex filifolia	0.17	0.12	0.12	0.17	0.00
Carex heliophila	0.00	0.00	0.13	0.11	0.00
Carex eleocharis	1.00	1.95	1.78	1.78	0.48
Per cent density total grasses and sedges	13.37	13.23	11.50	11.18	7.67
Forbs:					
Selaginella densa	8.70	6.41	5.13	4.20	0.00
Artemisia frigida	2.01	0.93	0.91	0.84	0.36
Phlox Hoodii	0.13	0.51	0.29	0.36	0.58
Artemisia cana	0.80	0.12	0.30	0.00	0.00
Eurotia lanata	0.00	0.00	0.00	0.00	0.15
Rosa Macounii	0.16	0.03	0.08	0.03	0.00
Number of sites	4	5	7	12	6

TABLE 6.—CORRELATION COEFFICIENTS FOR FOUR GRASSES WITH CLAY CONTENT

Variates Per cent clay in soil and grass cover in percentage	Correlation coefficient	Required for significance		Remarks
		One per cent level	Five per cent level	
Per cent clay and *Stipa comata*[*]	0.109	0.478	0.374	Not significant
Per cent clay and *Koeleria cristata*	0.178	0.418	0.325	Not significant
Per clay clay and *Bouteloua gracilis*	−0.458	0.478	0.374	Significant
Per cent clay and *Agropyron Smithii*	0.895	0.418	0.325	Highly significant

[*] Two pairs of variates.

represents a large percentage of the total vegetation cover on all soils except heavy clay. The dominant forb for the area is *Artemsia frigida*, which appears in various proportions on all soil textures. It does not appear to be correlated with texture, and dense stands are indicative of an overgrazed condition in the northern Great Plains area. *Phlox Hoodii*, *Malvastrum coccineum*, *Haplopappus spinulosus*, and *Rosa Macounii* are common forbs of the area.

Table 5 presents a summary of the data that were collected during the study. The correlations as established are fairly evident from these data.

CONCLUSIONS

The correlation that exists between soils and vegetation is difficult to analyse because of the effects of climate. For this paper, climatic conditions have been assumed to be similar over the study area.

A close correlation was found to exist between *Agropyron Smithii* and per cent clay content of the soil. Consequently, soils possessing sixty per cent of clay and over were dominated by *Agropyron Smithii* and *Stipa viridula*. These grasses are indicative of clay soils or, more truly, of soil moisture conditions associated with clay.

Bouteloua gracilis and per cent clay in the soil showed a significant negative correlation. *Koeleria cristata* and *Stipa comata* showed no significant correlation with per cent clay content of the soil.

On loam soils, however, *Stipa comata* appears to be the dominant grass, yielding in the lighter soils to *Bouteloua gracilis* as the dominant, and in the heavier soils to *Agropyron Smithii*.

It is believed from information obtained during this study that there is a definite correlation between soil, plants, and climate. Much work to date, however, has been based on visual analysis which appears to be true for large areas, e.g., the shortgrass formation or the mid-grass formation. However, in small areas where more intensive study is possible, it appears that many correlations based on visual analysis are not statistically sound. For example, *Stipa comata* seems dominant on loam soils and yet there is no significant correlation between *Stipa comata* and the per cent clay content of the soil.

It is generally conceded, for a limited area at least, that much can be determined about the vegetation by examining the soil. This usually gives a good indication of climatic conditions that exist and the type of vegetation that would persist under the existing conditions of climate and soil.

Some plants may be used as indicators. *Artemisia cana* in most instances indicates a light well drained soil. *Eurotia lanata* is most prevalent on the heavier soils, while *Rosa Macounii*, where very abundant, indicates sandy soil recently stabilized. Of the grasses, *Agropyron Smithii* indicates soils heavy in texture with high water holding capacity, while *Bouteloua gracilis* is more prevalent on light textured soils. *Distichlis stricta* (Torr.) Rydb. indicates soils high in salt concentrations, while *Calamovilfa longifolia* and *Oryzopsis hymenoides* (Roem. & Schult.) Ricker indicate sand.

It is evident from the information obtained and the data that are available that more intensive work is needed in the field of soil, plant, and climate relationships.

SUMMARY

The approximate centre of the area studied is Swift Current, Saskatchewan. Of the total area, approximately sixty per cent is under cultivation. Of the remainder, two per cent is abandoned land, dominated by perennial and annual weeds, and thirty-five to forty per cent is native sod.

The climate is classified as semi-arid with a precipitation-evaporation ratio of 0.45. The highest rainfall occurs during the month of June. Great extremes of temperature characterize the area, with a total variation of over 150 degrees, and a frost-free period of approximately 120 days.

Composite soil samples were collected and analysed from all sites selected. Mechanical analysis, moisture equivalent, and wilting coefficient were obtained. Close correlation between the size of soil particles and the moisture factors is shown.

The vegetation of the region is described. This is comprised mainly of the grasses of the mid-grass formation, made up of several associations, namely, *Stipa-Bouteloua*, *Bouteloua-Stipa*, *Agropyron-Stipa*, and an *Agropyron* consociation. For the most part, the soils of the zone are characterized by light-brown coloured surface soils and a layer of lime ($CaCO_3$) accumulation relatively close to the surface.

The mineral elements of the soil do not appear to be limiting factors of plant growth on the area, although responses have been obtained by the use of manure. To date, however, there is little, if any, information on the mineral requirements of native plants in the Brown soil zone.

Climatic conditions throughout the area are assumed to be the same; however, soil texture which indirectly controls available moisture, is definitely correlated with the dominant grasses. A highly significant correlation was found between *Agropyron Smithii* and per cent clay content of the soil; and a significant negative correlation was observed between *Bouteloua gracilis* and the per cent of clay. However, no correlation was found between *Koeleria cristata* or *Stipa comata* and the per cent clay content of the soil.

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FACTORS IN RELATION TO TUBER QUALITY IN POTATOES

I. PENETRATION FORCE AND ELECTRICAL RESISTANCE[1]

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INTRODUCTION

The Irish potato (*Solanum tuberosum* Linn.) has been the subject of extensive tests of a clinical nature to determine quality for human food and other uses. The popular and very important nature of the vegetable has resulted in great interest in such "quality" tests; and in recent years certain of the tests have been used rather widely by plant breeders in order to get approximate starch content at an early stage in the development of promising seedlings.

Certain disadvantages of (1) panel judging of cooked tubers, (2) specific gravity of tubers, (3) alcohol tests and (4) quantitative starch determination, have led to attempts by at least two workers [Dallyn (5) and Turnquist (11)] to determine the feasibility of other means of measuring the density of potato tubers, or to measure some factor other than density that might be related as directly to tuber density as the latter is to starch content (3, 4, 7). Bewell (2) and Smith and Nash (10) have demonstrated a high correlation between mealiness and dry matter content and Rinear (9) has shown that dry mealy potatoes are preferred by 71 per cent of 1,653 consumers. It might also be of interest to note that in his survey by Rinear, 0.89 per cent of the consumers preferred watery or moist potatoes and only 0.6 per cent mentioned flavour as a quality criterion.

According to Gilmore (6) potato tuber dry matter content may vary rather widely (21.1 per cent to 27.2 per cent) within a variety, but other investigators (1, 3, 5) have shown that even wider variability is encountered between varieties. This indicates that, although a fairly large number of tubers of any one variety must be used in a test to ensure a representative sample, nevertheless any test that will measure tuber density, or correlated factors, can be used to separate one variety from another in this characteristic.

This paper discusses two attempts to secure results similar to those achieved by the well-known brine immersion tests, based upon tests that are less time-consuming or that require less cumbersome equipment.

EXPERIMENT 1 (Penetration Force Trials)

For many years maturity in tree fruits had been estimated by means of a "pressure gauge" that measures flesh firmness. It seems reasonable to assume that tubers of different density should differ in their resistance to an applied force. If such a test proved to offer a reliable guide to tuber density, it would be quick and convenient and would do little damage to tubers that were desired for other tests or for seed purposes. The value of such a test would be proved if a significant correlation between "penetration force" and density could be established, not only within a variety but regardless of variety.

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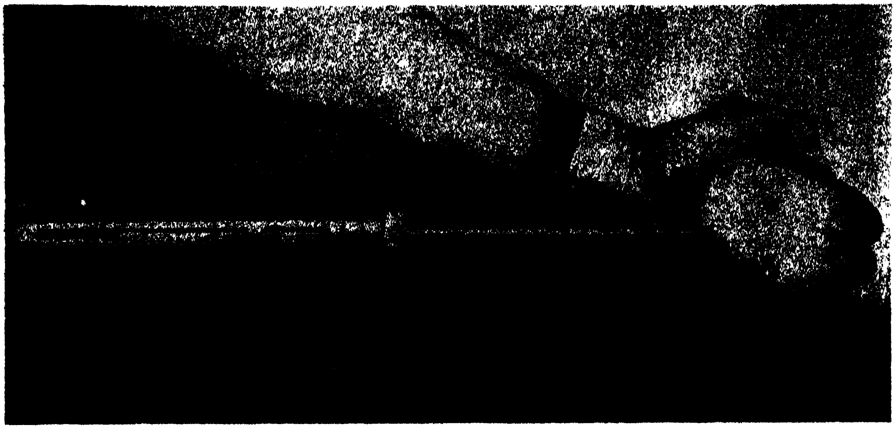


FIGURE 1. Method of using the pressure tester for penetration of potato tubers. The force exerted is measured directly in lb. pressure.

Materials and Methods

Potato tubers, the density of which has been determined by immersion in brine solution of known specific gravity, were selected at random from ten different varieties. A Ballauf Pressure Tester, designed for testing maturity, was fitted with a specially adapted plunger of 5 mm. diameter. The penetration apparatus is shown in Figure 1.

The plunger was forced into tubers of uniform size to a depth of 34 mm., care being taken to avoid regions at eyes, where cortical thickness variations would be encountered. Preliminary tests showed that the removal of the skin did not influence the readings, so no tuber paring was done in the test reported here. Seven penetrations were made on each tuber, and these readings were averaged to give a "mean force" record for each tuber.

Results

The results of a total of 196 penetration readings are summarized in Table 1.

TABLE 1.—PENETRATION FORCE READINGS ON POTATO TUBERS OF SEVERAL DENSITIES

Specific gravity classes	No. of tubers tested	Mean force reading*	Range of tuber force readings
1.065	1	9.11	8.8–9.5
1.075	5	8.48	7.5–9.5
1.080	3	8.87	8.0–9.8
1.085	3	8.32	7.8–9.3
1.090	3	8.85	8.5–9.3
1.095	4	8.97	8.5–9.0
1.100	4	8.41	8.0–9.0
1.105	4	8.23	7.8–9.0
1.110	1	7.86	7.5–8.3
Total	28	8.57	Av. 8.0–9.2

* These values are empirical only, but are quite suitable for comparative purposes.

The specific gravity-penetration force correlation coefficient was computed and found to be 0.226. This figure does not represent significance.

Discussion

The penetration force, in so far as determined by this experiment, has no close relationship with tuber density. Apparently, the flesh firmness of potato tubers is dependent upon internal factors other than, and presumably not related to, dry matter content. This result is in accord with that of Turnquist (11) whose brief report in 1948 represents the only published record of a test of this nature that has been noted by the authors. Dallyn (5) in an unpublished 1947 report, recorded similar findings, but both Dallyn and Turnquist determined that there were significant differences in tuber flesh firmness between varieties. The latter also found a difference due to locations and a significant interaction between locations and varieties.

Because of the direct or the indirect relationship that is suggested by the fact that varieties themselves exhibit definite differences in tuber firmness, it is suggested that further penetration experimental work be undertaken. There is some possibility that a relationship can be shown to exist between potato storage adaptability and flesh firmness.

EXPERIMENT 2 (Electrical Resistance Trials)

Since dry matter and specific gravity are positively correlated an inverse relationship can be postulated—one between moisture content and specific gravity. A widely used method of measuring the moisture content of cereal grains is by grinding them and subjecting the finely divided material to a test for electrical conductivity. Similar means are used for determining moisture content of dehydrated fruit and vegetable stock. It was decided to attempt a similar test upon uniform sections of fresh potato tubers.

Before deciding upon what constitutes a uniform tuber section, the structure and composition of a typical tuber must be considered. Chatfield and Adams (3) give a variation of 66.0 per cent to 85.2 per cent for tuber moisture content, with a mean of 77.8 per cent. Ash content varies from 0.5 per cent to 1.9 per cent with a mean of 1.0 per cent. Many of the ash constituents are in non-ionizable form and hence have poor electrical conductivity, and for this reason the resistance to the passage of electrical current, rather than the conductivity, was assumed to be the more practical measurement.

An instrument used for measuring electrical resistance is the ohmeter. It is compact and relatively inexpensive. If the ohmeter method of measuring such resistance could be shown to have value, it would be simple and rapid.

Since the potato tuber is a modified, very much thickened and shortened stem, with few or many nodes, and with definite layers of tissue somewhat corresponding to those of normal stems, it can be assumed that the tuber composition is not homogeneous. This lack of homogeneity is shown in Figure 2.

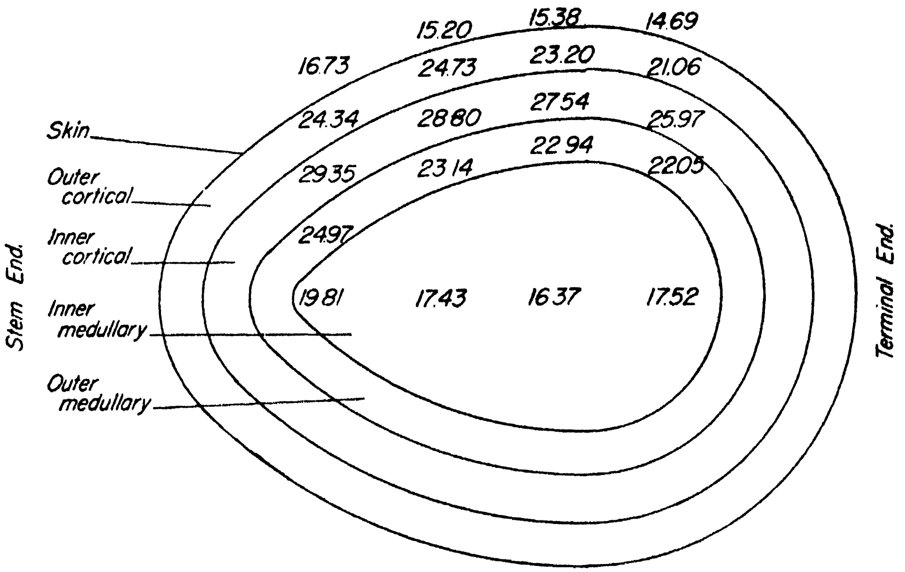


FIGURE 2. Variation in percentage of dry matter from samples of 20 tubers. (After Jackson and Glynne (8))

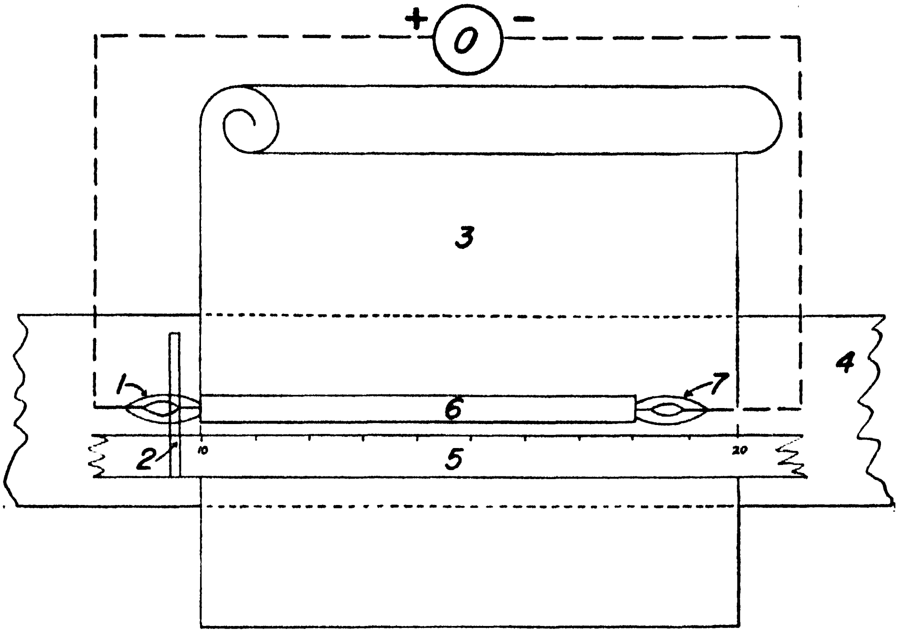
This figure shows that dry matter varies from the stem end to the terminal end, as well as within the several regions. Thus, skin and inner medullary (pith) regions are relatively low in dry matter while the cortical regions are relatively high. The outer medullary area (vascular tissue) is intermediate in dry matter content. From the above it will be seen that a flesh sample must be chosen carefully if the measurements are to represent the whole tuber.

While divisions into quarters or eighths and selecting diagonally opposite sectors will give the truest representation of a tuber (8), this is not feasible for the resistance measurements since the mass to be measured must itself be easily measurable, and of uniform diameter. It was therefore decided to take diagonal cores from the tubers for the work reported here.

Materials and Methods

The cores of tubers of known specific gravity were taken from ten different varieties, and as in Experiment 1, no attempt was made to keep the varieties separate. Rather, care was taken to secure specimens showing a wide range of tuber density, whatever the variety. The apparatus was set up as illustrated in Figure 3.

A Simpson ohmeter was used. The apparatus is explained in the diagram. The waxed paper strip was used between the baseboard and the meter-stick to prevent wetting the board, which would reduce resistance readings. The ohmeter anode was fixed at the 10 mm. mark and sections from tubers were removed by means of a No. 4 cork borer. Tuber eyes were avoided, and all borings were taken so that the section length was equal to the mean diameter of the tuber. This measure was calculated by



1. ANODE 2. CELLULOSE TAPE 3. WAXED PAPER
4. BASEBOARD 5. METRE STICK 6. TUBER CORE
7. CATHODE

FIGURE 3. Arrangement of apparatus for measuring resistance in potato tubers.

taking the average of the length, breadth and depth. The skin at the core ends was peeled away before resistance readings were taken.

The section was laid beside the measure on the waxed paper and with one end touching the fixed anode terminal. When the cathode was applied to the other end the resistance in ohms was read from the ohmeter and the section length was read from the measure. The waxed paper strip was moved after each measurement to ensure that the new section rested upon dry paper.

Results

The specific gravity classes, number of readings, average resistance in 1,000 ohms per linear centimetre of section and the range of resistance readings appear in Table 2.

Casual examination of these data presents no apparent trend in any direction, and this is borne out by the correlation coefficient of specific gravity/resistance, which was 0.008, or negligible.

Discussion

From the data presented herein, no association between specific gravity (tuber density) and electrical resistance is indicated. This is in spite of apparently logical reasoning that would suggest such an association. The diversity of the dry matter content within any tuber, as shown in

TABLE 2. ELECTRICAL RESISTANCE OF POTATO SECTIONS OF KNOWN DENSITY

Specific gravity classes	No. of readings	Average resistance (in 1000 ohms per cm.)*	Range of resistance
1.060	2	7.80	6.9–8.7
1.065	2	6.15	5.8–6.5
1.070	5	6.34	5.5–6.5
1.075	7	6.71	4.3–8.4
1.080	8	6.16	5.1–8.1
1.085	6	5.54	5.1–6.1
1.090	10	5.80	5.4–6.8
1.095	7	5.90	5.2–6.8
1.100	5	5.68	4.6–6.7
1.105	4	6.63	6.2–7.0
1.110	3	6.23	4.9–7.5
Total	59	Av. 6.27	

* Sections of variable length but all were 8.2 mm. in diameter.

Figure 2, indicates the difficulty of securing a representative tuber portion by the core method. Possibly if the resistance of the whole tuber could be measured, a positive relationship between the two characters might be demonstrated but since no two tubers are exactly the same size and shape this would necessitate maceration of the tuber. If this were done, the test would be of little practical use or significance.

SUMMARY

1. The desirability of more satisfactory technique for measuring tuber density in Irish potatoes is discussed. Tubers of several varieties whose density has been calculated by the brine immersion method were subjected to penetration force and electrical resistance tests.

2. Measurement of tuber quality by means of a fruit maturity gauge (fitted with a 5 mm. plunger), showed a specific gravity/penetration force correlation of $r = 0.226$, and this was not significant. A total of 196 penetration force readings were made.

3. Sections from 59 tubers, taken in such a way as to be as representative as possible of internal composition, were used in electrical resistance measurements. An ohmeter was used and readings were expressed in 1,000 ohms per cm. length of core. A correlation $r = 0.008$ showed no indication of a relationship between tuber density and electrical resistance.

ACKNOWLEDGMENTS

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A STUDY OF SOME SEED-BORNE DISEASES OF CEREALS IN CANADA

I. COMPARISON OF SEED TESTING METHODS[1]

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INTRODUCTION

The recent expansion of the seed-growing industry in Canada has increased the demand among those engaged in the seed trade for up-to-date information concerning germinability, disease, and treatment of seed. It has been recognized, for instance, that the standard germination test on blotters is inadequate because (1) it does not give a uniformly accurate index of the percentage of healthy seedlings that may be expected from a seed stock when sown in the field; (2) because it does not reveal the nature and importance of many of the defects present in or on the seed, and (3) because it does not show the extent to which a lot of seed will benefit from treatment.

A comprehensive study of three aspects of the seed-borne disease problem was undertaken by the Dominion Laboratory of Plant Pathology at Winnipeg, at Saskatoon, and at Edmonton. The study included, *first*, a comparison of methods used by these laboratories for testing cereal seed; *second*, a survey of the most common defects of cereal seed; and, *third*, a comparison of germinations and yields obtained by planting seed with different percentages of infected kernels, together with a study of the effect of weeds in experimental plots on the gain in stand and yield following seed treatment.

The results of co-operative experiments on these subjects are to be presented in serial fashion, the present paper being the first of the series.

This section of the study describes and compares several laboratory and greenhouse methods of testing cereal seed for germinability and for the seed-borne organisms (except smut fungi) which affect seedling development and yield of the mature plant. A comparison of these methods is made with respect to: (1) uniformity of results, when they were used by different investigators; (2) their reliability, when they were used in an attempt to forecast how seed lots will germinate and yield in the field, and how they will respond to seed treatment; (3) their usefulness in indicating the condition of the seed.

The data are presented mostly in the form of correlations between the results of tests with 90 samples of seed in 1942-43, and with 12 samples in 1943-44. The 90 samples of seed used in 1942-43 included wheat, oats and barley from Manitoba, Saskatchewan and Alberta—10 samples of each crop from each province—and they were taken at random from seed lots to be sown by farmers in those provinces in 1943. The 12 farm samples used in 1943-44 (also wheat, oats and barley) were selected especially to represent healthy, diseased, and frosted seed.

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COMPARISON OF SOIL AND INCUBATION TESTS

Methods

The non-sterile soil test described by Machacek and Wallace (2), and the incubation test described by Simmonds and Mead (3), were compared at all three laboratories. Briefly, the nature of the two tests was as follows:

In the soil test, two 100-kernel aliquots (one treated with Ceresan—5 per cent ethyl mercury phosphate—and the other left untreated) from each seed lot were sown in adjacent rows in a large bed of non-sterile soil in the greenhouse. After a growing period of 10 days at approximately 20° C., the seedlings were taken from the soil, counted, and examined individually for abnormalities or lesions on the stems and leaves. This examination showed the percentage of normal and abnormal seedlings and the percentage of seedlings attacked by seed-borne, root-rotting and leaf-spotting fungi, such as *Helminthosporium sativum* P. K. & B., *H. teres* Sacc., *H. avenae* Eidam, *Septoria nodorum* Berk., and *Fusarium* spp. In addition, the test showed the degree to which each seed lot was improved by treatment with Ceresan. Certain difficulties were recognized in conducting the soil test. These included differences in the soil, and variations in watering and temperature. The first two difficulties could not be overcome, whereas the third difficulty, maintenance of a uniform temperature, could be overcome only to a limited extent, as adequate greenhouse temperature control equipment was lacking.

In the incubation test, untreated seed was plated on moist filter paper in Petri dishes, 25 seeds to a dish, and the cultures were held constantly at 24° C. for four days. The seeds were then examined directly through a low-power binocular microscope, this examination providing data relating to the amount of lesioning of seedlings, seed contamination and decay by such fungi as *Helminthosporium* spp. and *Fusarium* spp., as well as data regarding percentages of normally and abnormally germinating seeds. The meanings given to normal germination and abnormal germination were of the sense used by the seed testing laboratories of the Canada (Department of Agriculture) Production Service (1). According to the rules laid down for seed testing, abnormal germination or abnormal sprouts means seedlings with improperly developed shoots or roots. Among the abnormalities recognized are: watery or glassy, coiled, twisted, swollen, prematurely split, or extremely spindly shoots; stunted or swollen roots, or roots lacking normal growing points or root hairs.

The authors did not have an opportunity to make a joint study of seedling material before the experiments were started. This circumstance may account for some of the disagreements appearing among the experimental results given below.

Comparison of Data on Germination

The recorded data on normal and abnormal germination and lesioning from untreated seed sown at the three laboratories are summarized in Table 1. The data given are averages for 30 samples each of wheat, oats and barley, portions of the same samples of each crop having been tested at each laboratory.

TABLE 1.—COMPARISON OF AVERAGE DATA OBTAINED BY TWO SEED-TESTING METHODS (SOIL TEST AND INCUBATION TEST) AT THREE LABORATORIES

Data compared	Crop	Method	Testing laboratory		
			Winnipeg	Saskatoon	Edmonton
			%	%	%
Normal germination	Wheat	Soil	64.6	81.8	73.6
		Incubation	89.1	87.0	93.2
	Oats	Soil	60.8	79.7	75.4
		Incubation	70.8	51.2	57.9
	Barley	Soil	81.8	89.2	84.1
		Incubation	86.2	63.4	61.8
Abnormal germination	Wheat	Soil	10.7	1.5	5.1
		Incubation	4.7	8.7	1.0
	Oats	Soil	8.1	2.1	11.1
		Incubation	3.5	28.4	9.3
	Barley	Soil	4.9	1.3	2.3
		Incubation	2.3	9.5	4.8
Lesioning	Wheat	Soil	3.3	1.3	9.7
		Incubation	2.5	1.3	—[1]
	Oats	Soil	4.7	0.7	3.2
		Incubation	3.7	0.2	—
	Barley	Soil	6.2	3.2	8.3
		Incubation	1.8	4.0	—

[1] Lesioning in the incubation test was not recorded at Edmonton.

The data in Table 1 indicate that there was considerable variation among the readings obtained by the two seed-testing methods at the three laboratories. Normal germination, for instance, tended to be higher in all three crops in the incubation test at Winnipeg than in the soil test. In contrast, at Saskatoon and Edmonton such a trend was observed only in wheat, but there was an opposite trend in oats and barley. Abnormal germination at Winnipeg was consistently higher in the soil test than in the incubation test, at Saskatoon it tended to be higher in the incubation test, and at Edmonton it was variable. Data on lesioning were incomplete, no definite trend being observed.

The experimental data obtained during this comparison of testing methods were subjected to analyses of correlation. The numerous coefficients of correlation thereby obtained are tabulated in Tables 2, 3, 4, 5, 6, and 7. Those coefficients applying to normal and abnormal germination are given in Tables 2, 3, and 4; those applying to lesioning are given in Tables 5, 6, and 7.

As Table 2 shows, fairly good agreement between the two tests, when data on normal germination were correlated, was obtained for oats at Winnipeg, for wheat at Saskatoon, and for wheat and oats at Edmonton. Otherwise there was poor agreement. In the case of abnormal germination, correlation coefficients were either low or negative, thus indicating very poor agreement between the two sets of data.

When the data from the soil tests made at the three laboratories were correlated, it was found (Table 3) that agreement was good in the case of normal germination, but fair or poor in respect to abnormal germination.

The reason for the latter disagreement is not known. It is suggested that the three interpretations of the class "abnormal germination" did not coincide.

TABLE 2.—CORRELATIONS OF DATA ON NORMAL AND ABNORMAL GERMINATION IN THE SOIL AND THE INCUBATION TEST

Laboratory	Coefficients of correlation					
	Normal germination			Abnormal germination		
	Wheat	Oats	Barley	Wheat	Oats	Barley
Winnipeg	0.183	0.646	0.273	0.073	−0.250	0.217
Saskatoon	0.610	0.265	0.244	0.000	−0.002	0.137
Edmonton	0.405	0.629	0.136	−0.137	−0.254	0.150

Significance: 5 per cent, 0.361; 1 per cent, 0.436.

TABLE 3.—CORRELATIONS OF DATA ON NORMAL AND ABNORMAL GERMINATION OBTAINED FROM SOIL TESTS AT THREE LABORATORIES

Laboratories compared	Coefficients of correlation					
	Normal germination			Abnormal germination		
	Wheat	Oats	Barley	Wheat	Oats	Barley
Winnipeg-Saskatoon	0.805	0.837	0.832	−0.205	0.612	0.055
Winnipeg-Edmonton	0.639	0.919	0.585	0.422	0.474	0.105
Saskatoon-Edmonton	0.588	0.966	0.462	0.202	0.118	0.182

Significance: 5 per cent, 0.361; 1 per cent, 0.436.

Agreement between laboratories on normal germination in the incubation test was good, except in the Winnipeg-Saskatoon and Saskatoon-Edmonton comparisons with oats (Table 4). Correlations between the data on abnormal germination was low in seven out of nine comparisons, the coefficients being significant only in the Saskatoon-Edmonton comparison for oats and in the Winnipeg-Edmonton comparison for barley.

TABLE 4.—CORRELATIONS OF DATA ON NORMAL AND ABNORMAL GERMINATION OBTAINED IN INCUBATION TESTS AT THREE LABORATORIES

Laboratories compared	Coefficients of correlation					
	Normal germination			Abnormal germination		
	Wheat	Oats	Barley	Wheat	Oats	Barley
Winnipeg-Saskatoon	0.933	0.107	0.691	0.000	−0.287	0.065
Winnipeg-Edmonton	0.488	0.456	0.606	0.241	−0.133	0.478
Saskatoon-Edmonton	0.494	0.001	0.819	0.105	0.477	0.066

Significance: 5 per cent, 0.361; 1 per cent, 0.436.

Comparison of Data on Lesioning

The percentage of seedlings bearing lesions on the coleoptile or roots was recorded at the time germination was determined. Data concerning lesioning in the incubation test, however, were obtained at only two laboratories, Winnipeg and Saskatoon.

It was found that there was good agreement between the two tests on lesioning for wheat at Saskatoon, for oats at Winnipeg, and for barley at both laboratories, and that there was poor agreement for wheat at Winnipeg, and for oats at Saskatoon (Table 5).

TABLE 5.—CORRELATIONS OF DATA ON LESIONING FROM SOIL AND INCUBATION TESTS AT TWO LABORATORIES

Laboratory	Coefficients of correlation		
	Wheat	Oats	Barley
Winnipeg	0.068	0.471	0.784
Saskatoon	0.910	−0.181	0.939

Significance: 5 per cent, 0.361; 1 per cent, 0.436.

Correlation between the results on lesioning in soil at the three laboratories was high on wheat, moderate but significant on barley, and low or negative on oats (Table 6).

TABLE 6.—CORRELATIONS OF DATA ON LESIONING FROM SOIL TESTS AT THREE LABORATORIES

Laboratories compared	Coefficients of correlation		
	Wheat	Oats	Barley
Winnipeg-Saskatoon	0.898	−0.109	0.643
Winnipeg-Edmonton	0.782	0.211	0.397
Saskatoon-Edmonton	0.704	−0.250	0.435

Significance: 5 per cent, 0.361; 1 per cent, 0.436.

In the incubation tests made at the Winnipeg and Saskatoon laboratories, there was good agreement on lesioning of wheat and barley, but no agreement in the case of oats (Table 7).

TABLE 7.—CORRELATIONS OF DATA ON LESIONING FROM INCUBATION TESTS AT TWO LABORATORIES

Laboratories compared	Coefficients of correlation		
	Wheat	Oats	Barley
Winnipeg-Saskatoon	0.660	−0.105	0.939

Significance: 5 per cent, 0.361; 1 per cent, 0.436.

Comparison of Laboratory Data with Those Obtained Under Field Conditions

Eighteen representative samples of seed, six each of wheat, oats and barley, were sown in the field for the purpose of obtaining a comparison of field data with data obtained in the greenhouse (soil test) and in the laboratory (incubation test). After these data were obtained, they were combined and correlated with the combined data from the soil test and the combined data from the incubation test. The correlation coefficients obtained with these data are shown in Table 8. It may be seen that there was better agreement between the soil test and the field test than between the incubation test and the field test at all three laboratories.

TABLE 8.—A COMPARISON OF NORMAL GERMINATIONS IN THE SOIL TEST AND THE INCUBATION TEST WITH THAT OBTAINED IN THE FIELD

Laboratory	Coefficients of correlation	
	Soil test and field	Incubation test and field
Winnipeg	0.921	0.593
Saskatoon	0.855	0.287
Edmonton	0.681	0.073

Significance: 5 per cent, 0.468; 1 per cent, 0.590.

Comparison of Seed Treatment Data

In order to determine whether the gain in germination, obtained in the soil test following treatment of the seed with Ceresan (5 per cent ethyl mercury phosphate), is reflected by a gain in yield under field conditions, 12 samples of seed were sown, treated and untreated, in the greenhouse and field at each of the three stations concerned with this investigation. A correlation of the changes in germination with the changes in yield gave the coefficients shown in Table 9. According to these coefficients, the gain in normal germination in the soil test had little or no relation to the yield under field conditions.

TABLE 9.—CORRELATION OF THE CHANGES INDUCED BY SEED TREATMENT IN THE PERCENTAGE OF NORMAL SEEDLINGS IN THE SOIL TEST WITH THE CHANGE IN YIELD INDUCED BY SEED TREATMENT UNDER FIELD CONDITIONS

Laboratory data from:	Field data from:	
	Manitoba	Saskatchewan
Winnipeg	0.230	0.274
Saskatoon	0.143	0.331
Edmonton	0.450	−0.200

Significance: 5 per cent, 0.707; 1 per cent, 0.834.

Summary

An examination of data on normal germination obtained from laboratory tests failed to show a consistent agreement between laboratories when they used the soil test and the incubation test on the same seed. This lack of agreement could have arisen from an unfamiliarity of the investigators with one or both of the tests. When the data from the soil test alone were considered (Table 3), there was, for three cereals, a high correlation between percentages for normal germination obtained at the three laboratories. It was shown (Table 8) that these results agreed fairly closely with those from a field test, indicating that the soil test was reasonably reliable for the purpose of forecasting germination in the field. Agreement between the three laboratories was also fairly good, except on oats, when the incubation test was used to measure normal germination in the laboratory (Table 4), but this test did not appear to be consistently reliable as a means of indicating germination in the field (Table 8).

COMPARISON OF DATA FROM SOIL AND SAND TESTS IN PAPER BOXES

Methods

In further studies, non-sterile soil and sand were used to test 12 selected samples of wheat, oats, and barley. These tests were run concurrently at the three laboratories, under conditions as similar as possible. The soil and washed coarse sand were placed in separate cardboard boxes (4½ in × 4½ in. × 1½ in. and with perforated bottoms) to the depth of one-half inch. One hundred seeds were sown and spaced evenly in each box on the basal layer of the medium and then were covered with another half-inch layer of the same medium. The boxes were planted in quadruplicate, arranged at random on metal trays, and watered uniformly then, and when necessary later. These tests were carried on for 10 days at about 20° C., and after this interval the seedlings were removed from the medium to be classified.

Comparison of Data on Germination and on Lesioning

The data on normal and abnormal germination obtained from wheat, oats, and barley sown in boxes, were combined and these combined data, together with similar data on lesioning, are presented in Tables 10, 11, 12, and 13. Table 10 shows the average percentages for normal germination, abnormal germination, and lesioning obtained at the three laboratories by the two tests. Tables 11, 12, and 13 show the correlation coefficients obtained after a statistical comparison was made.

TABLE 10.—AVERAGE PERCENTAGES OF NORMAL GERMINATION, ABNORMAL GERMINATION, AND OF LESIONING FOR 12 SAMPLES OF CEREAL SEED[1] SOWN IN SOIL OR SAND IN CARDBOARD BOXES

Data compared	Kind of test	Laboratory		
		Winnipeg	Saskatoon	Edmonton
		%	%	%
Normal germination	Soil	56.8	60.2	74.5
	Sand	48.0	59.5	72.0
Abnormal germination	Soil	13.0	11.6	4.8
	Sand	9.5	6.2	4.5
Lesioning	Soil	8.8	8.2	7.2
	Sand	27.0	19.4	10.8

[1] Three of these samples were damaged by frost.

The data shown in Table 10 indicate a considerable difference in the amount of abnormal germination and lesioning obtained by the two tests, and also a considerable variation in the normal germination noted by the three laboratories.

Correlations of test data showed that, at each of the three laboratories, there was a close relationship between the data obtained by the two tests (soil and sand) with respect to normal germination and lesioning. For lesioning this close relation held good only for Winnipeg and Edmonton.

TABLE 11.—CORRELATIONS OF DATA ON NORMAL GERMINATION, ABNORMAL GERMINATION, AND LESIONING OBTAINED IN SOIL-BOX AND SAND-BOX TESTS

Laboratory	Coefficients of correlation		
	Normal germination	Abnormal germination	Lesioning
Winnipeg	0.605	0.857	0.758
Saskatoon	0.778	0.345	0.924
Edmonton	0.883	0.978	0.698

Significance: 5 per cent, 0.576; 1 per cent, 0.708.

When soil was used as a testing medium, there was good agreement between laboratories in the amount of normal germination and fair agreement in respect to lesioning and abnormal germination (Table 12).

TABLE 12.—CORRELATIONS OF DATA REGARDING NORMAL AND ABNORMAL GERMINATION AND LESIONING IN SOIL-BOX TESTS

Laboratory data compared	Coefficients of correlation		
	Normal germination	Abnormal germination	Lesioning
Winnipeg-Saskatoon	0.943	0.579	0.371
Winnipeg-Edmonton	0.881	0.756	0.647
Saskatoon-Edmonton	0.945	0.481	0.652

Significance: 5 per cent, 0.576; 1 per cent, 0.708.

In the sand tests, better agreement between laboratories was obtained in respect to lesioning than in respect to germination, although the coefficients of correlation in all cases were significant (Table 13).

TABLE 13.—CORRELATIONS OF DATA REGARDING NORMAL AND ABNORMAL GERMINATION AND LESIONING IN SAND-BOX TESTS

Laboratory data compared	Coefficients of correlation		
	Normal germination	Abnormal germination	Lesioning
Winnipeg-Saskatoon	0.840	0.636	0.904
Winnipeg-Edmonton	0.672	0.878	0.848
Saskatoon-Edmonton	0.640	0.753	0.976

Significance: 5 per cent, 0.576; 1 per cent, 0.708.

Comparison of Box Data with Field Data

Seed of the 12 samples which had been tested in soil and sand in boxes in the greenhouse were also used in field tests. The purpose of these field tests was to compare the response of the samples (increase in normal germination) to treatment with Ceresan in the boxes under laboratory conditions with the response (effect on yield) under field conditions. The coefficients of correlation obtained in comparisons made at three laboratories are shown in Table 14.

TABLE 14.—COEFFICIENTS OF CORRELATION FOR A COMPARISON OF THE EFFECT OF SEED TREATMENT ON NORMAL GERMINATION IN SOIL AND SAND-BOX TESTS AND ON YIELD IN FIELD TESTS AT THREE LABORATORIES

Laboratory test	Field data from		
	Winnipeg	Saskatoon	Edmonton
Soil in boxes	0.300	−0.198	−0.262
Sand in boxes	−0.148	−0.290	−0.167

Significance: 5 per cent, 0.576; 1 per cent, 0.708.

These results show that there was either a low positive correlation, or a negative correlation, between normal germination in laboratory tests and the yield from field tests. It may be assumed, therefore, that increases in normal germination obtainable in box tests following treatment of the seed would not be necessarily an indication of a probable beneficial effect of seed treatment on yield.

Summary

Correlations of the data from soil-box and sand-box tests indicate that fairly good agreement was reached with respect to normal germination and lesioning when each laboratory ran parallel tests. The results were sufficiently close to suggest that either medium could be used in testing seed for germination and the presence of certain kinds of disease, such as rootrot, seedling blight, or seed rotting. There was no indication, however, that the response in these laboratory tests of a seed sample to seed treatment, as measured by germinability, would be evident in a test of yield in the field.

DISCUSSION

Previous experience of the authors has shown that the standard germination test used for cereals is unsuitable for the detection of their seed-borne diseases. It seemed apparent that more information about a seed sample could be secured by using a different type of test and this view is supported by the results of the experiments described above. In the present experiments, soil in a large bed or in cardboard boxes, sand in cardboard boxes, and moist filter paper in Petri dishes, were substituted for the germination blotters used in the standard germination test for wheat, and notes were taken on normal and abnormal germination, lesioning, seedling blight, seed decay, and response to seed treatment.

A study of correlation between the data obtained by the various tests showed that, when measuring normal and abnormal germination, each of the three investigators obtained in some cases quite different results from the greenhouse soil test than from the incubation test. This lack of uniformity suggests that one or both of these tests have weaknesses which affect their usefulness. When, however, the data from the greenhouse soil test and the incubation test were examined separately, it was found that agreement on normal germination between laboratories was better in the former test; but, in the matter of abnormal germination, agreement was poor in the soil test, and very poor in the incubation test. In respect to lesioning, agreement was good in both tests for wheat and barley, but very poor for oats.

From these results, it appears that the two tests were imperfect in certain respects, a few of which are as follows: (1) lesioning in the soil test was somewhat erratic, being affected by differences in soil and by variations in watering and temperature; (2) some fungi, such as *Helminthosporium teres*, did not regularly cause basal lesioning of plants in soil, and an extra test, on agar, has to be conducted to demonstrate their presence; (3) oats grew very poorly on moist filter paper in Petri dishes; and (4) moulds tended to spread in the Petri dishes.

The usefulness of the soil test was established in predicting the percentage of normal seedlings that may be expected from seed sown in the field, for, when a comparison was made of data from laboratory and field tests, it was found that, at all of the three laboratories, the results from greenhouse soil tests agreed very well with those from field tests. On the other hand, agreement of the incubation test data with field data was very poor. In addition, the soil test showed that certain samples, if treated with Ceresan, improved in normal germination, but this improvement was not followed consistently by an improvement in yield in field plot tests. Actually, very little relation was found between the results of the greenhouse tests and those of the field tests in respect to yield.

Certain criticisms of the greenhouse soil test and the incubation test with respect to applicability to laboratory procedure, ease and speed of operation, and freedom from contamination, were made by one or more of the authors. The possibility of using large beds of soil in a seed testing laboratory was questioned. Although this requirement (large beds of soil in a greenhouse) cannot be fulfilled in the existing official seed testing laboratories, it might be in future laboratories. There were indications that fungus contamination of seedlings could come from the soil. One of the authors felt that the incubation test was tedious and slow, and all of them agreed that moulds could contaminate the Petri dishes and affect adversely the germination of the seed. Favourable comments on these tests included the recognition that a large number of seed samples could be rapidly handled in the greenhouse soil test, which was said to be a natural test and one more closely related to actual growing conditions than an *in vitro* test or a test on blotters. The incubation test was recognized as one readily applicable to laboratory use and one in which the temperature could be controlled easily.

The laboratory tests with non-sterile soil, and washed non-sterile sand in cardboard boxes, yielded results on normal germination and lesioning for which a high degree of correlation was obtained, that is, the results were very similar for parallel tests in soil and sand at each laboratory. Furthermore, comparisons between these data showed good agreement between the three laboratories. The main criticisms of soil and sand in boxes were that it was difficult to keep them moist, and that, in the sand, fungi tended to spread from diseased seedlings to healthy ones.

As the best agreement between laboratories was obtained by the use of non-sterile soil or washed sand in cardboard boxes, and because this technique could be adapted to laboratory procedure (sand in boxes is used regularly for the germination of barley and oats), it is suggested that this method, with some modifications in temperature and moisture control, would be suitable for testing seed for seed-borne fungi. This conclusion is supported by the fact that, in some European laboratories, crushed brick is used as a germinating medium in seed-borne disease studies.

SUMMARY

In 1942-43, ninety samples of wheat, oats and barley were tested for germination and disease in non-sterile soil in the greenhouse and on moist filter paper in Petri dishes in a series of co-operative tests conducted at the Dominion Laboratories of Plant Pathology at Winnipeg, Saskatoon and Edmonton. Correlation studies of the data from these tests showed that the best agreement was obtained in the soil test. Both tests had commendable features, but they also had some faults which made them unsuitable for use or impracticable at the present time.

In a second series of tests, made in 1943-44, twelve samples of cereal seed were tested in non-sterile soil and in washed non-sterile sand contained in cardboard boxes. Agreement between the results of this limited series of tests from the three laboratories was much better than in the first series. Because of this improved agreement, and because this technique could be adapted to ordinary laboratory procedure, the suggestion is made that the latter type of test would be suitable for seed-borne disease studies, provided a standard medium was used.

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THE WHOLE EMBRYO METHOD OF TESTING BARLEY FOR LOOSE SMUT AS A ROUTINE TEST[1]

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The method commonly used to determine the presence of loose smut, caused by *Ustilago nuda* (Jens.) K. & S., in barley seed is to plant a sample of the seed and grow the plants until they come into head. The total number of healthy and of smutted heads are counted and the percentage infection is computed. Simmonds (1) showed that the presence of the mycelium of the fungus in the embryos of barley seeds may be detected by separating out the embryos and examining them with a microscope. Provided that, for any given seed lot of barley, the percentage of infected embryos is closely correlated with the percentage of smutted plants that is produced by the seed, an examination of the embryos could be substituted with advantage for the usual growth test as a means of determining the amount of loose smut that is likely to appear in a crop grown from the seed. The main advantages are that (*a*) the percentage of infected embryos present in a sample of barley seed may be determined much more quickly by the embryo test than by the growth test, and (*b*) no greenhouse or field plot space is required for the embryo test. If this test fulfils the purpose, it should be of considerable practical value to plant breeders and to those engaged in seed certification.

The present investigation was undertaken to ascertain whether or not the percentage infection, as determined by the embryo test, agrees closely with that obtained by growing a crop from the same seed stock.

MATERIALS AND METHODS

Most of the barley seed used in these experiments belonged to varieties which are in common use in Saskatchewan at the present time, or to varieties which have been grown to a considerable extent there in the past. However, the variety Montcalm predominated. Naturally-infected seed was secured in the first place from farmers and from experimental institutions, and infected samples were subsequently increased in experimental field plots.

Embryo tests were conducted by the Simmonds method (1), with minor changes in detail. Usually, all the embryos recovered from 500 seeds were examined, and, on the average, the number was approximately 475 embryos per sample.

Growth tests were conducted both in the greenhouse and in the field. In the former, the results were based usually on from one to two hundred plants. This number was much lower than is desirable, but it was limited by lack of space and poor light conditions that prevented some of the plants from producing heads. Field tests were conducted at two places in the province, Saskatoon and Indian Head. In the field tests ten eight-foot rows were devoted to each sample and about 80 plants matured heads in

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each row, making a total of approximately 800 plants at each place. Twenty-nine samples were sown at the former place and twenty-eight at the latter.

In the growth tests, all the heads affected with loose smut were collected and spores from each were germinated, in order to cull out any heads affected with false loose smut, caused by *Ustilago nigra* Tapke. Therefore, all data presented in this paper refer to heads known to be affected with *U. nuda*. The percentage infection in the different samples varied from 0 to 25, the average being 3 or 4 per cent.

COMPARISON OF PERCENTAGE INFECTION AS DETERMINED BY DIFFERENT TESTS

The results secured in the four different tests mentioned above are shown in Table 1. There was a high positive correlation between the percentages of infection determined by the visual examination of the embryos, on the one hand, and those determined by the several growth tests, on the other. The coefficient of correlation was over 0.97 in each case. The closeness of agreement between them is further illustrated by Figure 1.

TABLE 1.—THE PERCENTAGE INFECTION WITH LOOSE SMUT (*Ustilago nuda*), IN THIRTY SAMPLES OF BARLEY SEED, AS DETERMINED BY FOUR DIFFERENT TESTS

Variety	Sample designation	Embryo test	Greenhouse test	Field tests	
				Saskatoon	Indian Head
Montcalm	705	1.4	0.7	1.6	—
Montcalm	707	0.0	0.0	0.1	0.0
Montcalm	708	2.0	0.0	—	2.9
Montcalm	710	0.2	1.4	1.1	0.4
Montcalm	711	1.6	4.7	1.4	1.7
Montcalm	712	4.4	6.0	3.5	3.4
Montcalm	713	4.7	3.6	4.2	4.1
Montcalm	715	1.0	0.0	1.0	1.6
Montcalm	716	3.6	4.2	2.3	2.9
Montcalm	717	4.9	4.7	3.1	3.5
Montcalm	719	4.7	4.7	3.7	3.7
Montcalm	720	3.1	4.0	2.5	3.1
Montcalm	Balcarres	0.4	1.0	0.5	0.3
Montcalm	Kelvington	3 2	3.0	2.5	1.1
Montcalm	Melfort	1.3	3.0	0.5	0.6
Montcalm	Saltcoats	0.8	1.0	0.6	0.5
Montcalm	Tisdale	0.2	0.0	0.2	0.1
Montcalm	Codette	0.0	0.0	0.2	0.6
Plush	1947	1.2	1.0	1.0	0.7
Plush	1948	0.3	0.0	0.4	0.4
Velvon	Scott	0.0	0.0	0.0	0.1
Titan	Scott	0.0	0.0	0.1	0.0
Glacier	Border row	10.7	10.1	11.9	10.7
Glacier	Row 12	7.0	7.0	7.1	7.8
Glacier	Sealer 2	6.9	—	5.8	6.6
Multiple cross	17 per cent	24.4	22.8	19.8	—
Multiple cross	24 per cent	3.5	3.4	2.7	3.1
Multiple cross	2 per cent	0.2	1.2	0.6	0.3
Newal	Indian Head	6.2	4.2	7.0	6.6
Rex	Saskatoon	18.2	18.7	18.9	23.3

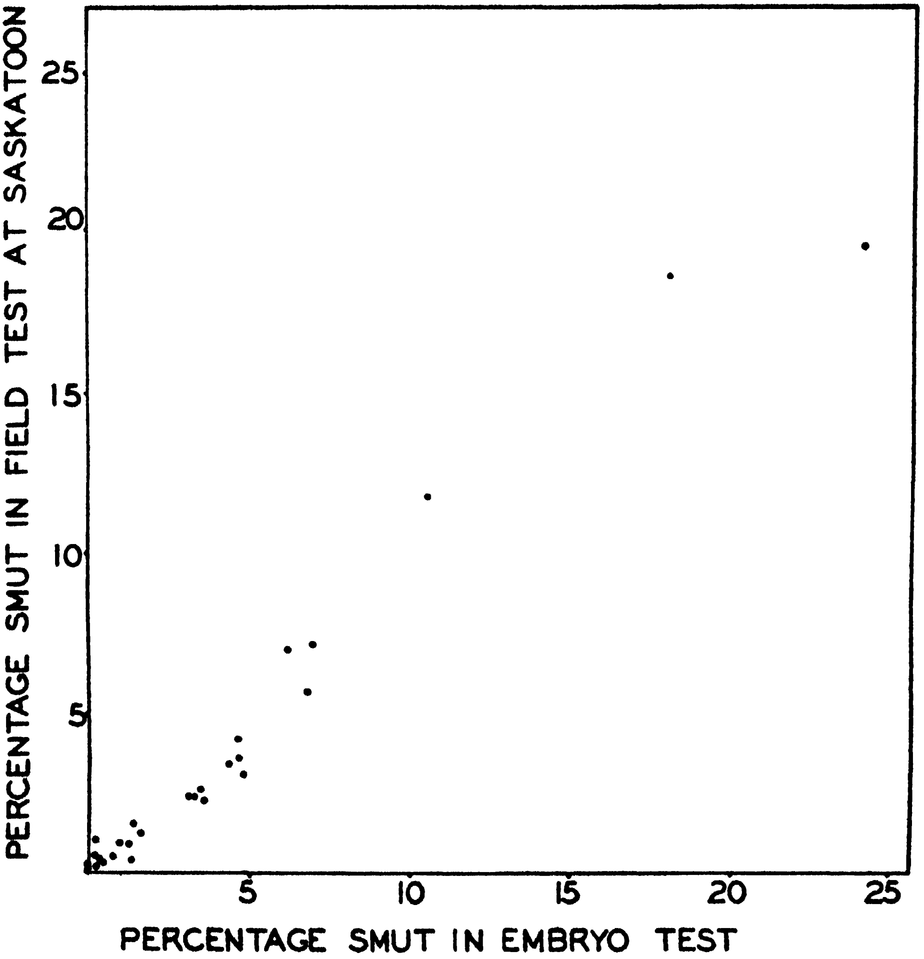

FIGURE 1. Graph showing the correlation between the percentage infection of 29 samples of barley seed, subjected to the embryo test, and the percentage infection arising in a growth test of the same samples conducted in the field at Saskatoon.

The average percentage infection found in the growth tests was practically the same as that determined by the embryo tests. In the 29 samples which were grown in the greenhouse, the average infection according to the embryo test was 3.77 per cent. In the growth test, it was 3.81 per cent. In 29 samples grown in the field at Saskatoon, the corresponding figures were 3.93 per cent and 3.60 per cent, and, for those grown at Indian Head, 3.22 per cent and 3.21 per cent. Both in the greenhouse and at Indian Head, where good conditions for germination prevailed, these percentages agree almost exactly. At Saskatoon, the soil in the field was so dry that a considerable portion of the seed did not germinate for several weeks after it was sown. Under these conditions, the percentage infection was somewhat lower than the embryo test indicated—3.60 per cent compared with 3.93 per cent.

DISCUSSION

The experimental data presented above show that, with the barley varieties used in this investigation, microscopic examination of the embryos of the seed gives a very good indication of what percentage of infection with loose smut may be expected when a crop is grown under normal conditions from the same sample. This conclusion may not be true for certain hypersensitive varieties, such as Titan (4), but it is true for most of the varieties used when they are infected naturally rather than artificially. Thren (2) has shown that the injury to the embryos caused by artificial inoculation with dry spores of *U. nuda* may be reduced by using small proportions of viable spores diluted with relatively large proportions of spores killed by artificial means.

It is possible that, on the average, embryos infected with *U. nuda* are somewhat weaker than non-infected embryos. They sometimes appear to be smaller and somewhat deformed. The fact that the average infection under field conditions at Saskatoon, as reported above, was less than the average infection shown by the embryo test supports this assumption. However, the mortality of the infected embryos, in the greenhouse and in the field at Indian Head, was not much greater than that of the non-infected ones. This is proven by the fact that the average infection at heading time corresponded closely to the percentage of infected embryos. In his work with loose smut of wheat, Vanderwalle (3) reports that he found no correlation between the presence of the fungus in the seed and the germinative vigour of the seed.

There is no doubt that the embryo test will prove useful whenever it is desirable to ascertain how severely a sample of seed barley is infected with true loose smut. Plant breeders can quickly determine whether or not *U. nuda* is present in the embryos of special seed which they intend to use. Government seed laboratory officials can use it as a basis for deciding whether or not to pass certain lots of seed for certification or registration. However, if the limit of tolerance allowed for this disease is very low, the usefulness of the test may be diminished. At the present time, a maximum of one per cent of smutted heads is allowed in fields producing certified seed and only one-half of one per cent in fields producing registered seed. It is conceivable, then, that enough embryos might be examined to make the test of practical value as far as certified seed is concerned, but the number necessary in the case of registered seed might be too great to make the test feasible as a routine procedure. Table 2 shows how many embryos should be counted to give reasonable assurance that smut will be detected when the percentage infection is in the order of one per cent, and of one-half of one per cent.

According to these computations, if smut infection is present to the extent of one per cent, and a representative sample consisting of 600 embryos is examined, there are only two chances in a thousand of failing to detect the smut. However, if only one-half of one per cent of the seeds carry smut, and a sample consisting of 600 embryos is examined, there are about five chances in every hundred of failing to detect any smut.

Similar computations to those by which the figures in Table 2 were derived show that, if a stock of seed carried loose smut infection in 1 per

TABLE 2.—THEORETICAL PROBABILITIES OF NO SMUT APPEARING IN A GIVEN SAMPLE OF SPECIFIED SIZE, TAKEN FROM A POPULATION IN WHICH THE PERCENTAGE INFECTION IS ONE PER CENT, AND ONE IN WHICH IT IS ONE-HALF OF ONE PER CENT

Number of embryos in sample	Probability of no smut if infection is one per cent	Probability of no smut if infection is one-half of one per cent
100	0.3664*	0.6053
200	0.1343	0.3664
300	0.0492	0.2218
400	0.01803	0.1343
500	0.00661	0.0813
600	0.00242	0.0492
700	0.00089	0.02978
800	0.00033	0.01803
900	0.00012	0.01091
1000	0.00004	0.00661

* The first term of the expansion of $\left(\frac{99}{100} + \frac{1}{100}\right)^{100}$.

cent of the seeds, the examination of a sample consisting of 600 embryos would, in 38 per cent of the tests, result in the finding of more than 1 per cent, and the seed stock would be rejected when it should not be. However, very few seed stocks carry exactly one per cent of infected seeds and, the further the actual percentage is from the limit of tolerance (i.e. one per cent), the less likelihood would there be of making a wrong decision from the results of the embryo test. The same difficulty would arise in a growth test involving 600 plants.

At times, negative results from the embryo tests would be of considerable value. The entire absence of smut in a sample is highly significant, and shows that little or no infection exists in the seed stock from which the sample is taken.

In the production of either certified seed or registered seed, it is felt that the embryo test would provide a better criterion than the present method for deciding what barley seed should be passed for certification and what should be rejected on account of the presence of loose smut. The present method relies on field counts made in the standing crop from which the seed comes. In the first place, the loose smut seen in the field may be partly false loose smut (*U. nigra*). The only way to make certain whether it is true or false loose smut is to germinate the spores from a number of the diseased heads. In the second place, the percentage of heads found infected with true loose smut in the standing crop may or may not give a true picture of the percentage of infected embryos in the seed produced by that crop. If environmental conditions are specially favourable for infection when the crop comes into flower, there is likely to be considerably more infection in the seed than was present in the standing crop. On the other hand, if conditions are very unfavourable for infection during the period that the crop is in flower, the percentage of infected seeds may be considerably less than the percentage of infected heads in the standing crop. Several instances were recorded in 1948 and 1949 in which the percentage infection in field plots in Saskatchewan was several times as high as the percentage infection in the seed produced on those plots. The embryo method of testing, as compared with the usual method, does not confuse false loose smut (*U. nigra*) with true loose smut (*U. nuda*), and it shows the amount of smut in the present generation, not the amount in the previous generation.

If the embryo test had been applied to the 30 samples dealt with in Table 1, and infection up to 1 per cent tolerated, then 19 of the samples would have been rejected and 11 passed for certification. In the field tests, 17 of these 19 samples showed over 1 per cent infection. Only two of the 11 samples produced over 1 per cent of infected plants. It is evident, then, that decisions relating to certification, based on these embryo tests, would have been correct 26 times out of 30, that is to say, if the percentages obtained in the growth tests were correct.

SUMMARY

The percentage infection with true loose smut, as found by a visual examination of the embryos of 30 samples of barley, is compared with the percentage infection shown in growth tests both in the greenhouse and in the field.

The agreement between the embryo test and the growth tests is very close, the coefficient of correlation being over 0.97 in each instance.

It is felt that the embryo test should prove valuable as a routine test, especially in connection with certain plant breeding work and the certification of seed barley.

ACKNOWLEDGMENT

The author is indebted to B. J. Sallans for the mathematical computations, including the data contained in Table 2.

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THE BREEDING VALUE OF SELECTED INBRED CLONES OF STRAWBERRIES WITH RESPECT TO THEIR VITAMIN C CONTENT[1]

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The strawberry breeding program at the University of Minnesota has included systematic inbreeding since 1922 and since 1929 has emphasized the selection of superior inbreds and their use in crosses. Selections from seedling progenies descended from standard varieties through one to three generations of selfing and selection have been used as parents in several experiments. The plants in one of these studies, a combining-ability experiment, fruited for the first time in 1948. Certain of the available populations were used in the present study of breeding behaviour with respect to the vitamin C content of the fruit.

The vitamin C content of strawberries is influenced by many factors, including sunlight, soil, and fruit size, maturity, and portion of the berry tested (2, 7). Berries grown in the shade, or upon shaded plants, contain less vitamin C than those grown in the sun, according to several workers (5, 9). Small berries have been found to have a higher concentration than large berries, and mature, deep red berries higher concentration than less mature, medium red berries (12).

Several workers (2, 3, 5, 7, 11,12) have reported large differences in vitamin C content among varieties, ranging from extremes of 41 mgm. to 142 mgm. of ascorbic acid per 100 gm. of fresh fruit. Some work has been done on the inheritance of vitamin C production in strawberries. Hansen and Waldo (6) in 1944 suggested that certain varieties were more influential than others when used as parents for high vitamin C, the Marshall, Clarke, and Progressive varieties and *Fragaria chiloensis* Duch. having the highest prepotency of the varieties and species studied. Slate and Robinson (12) in 1946 found a general positive relationship between the vitamin C content of the parent and the mean of its offspring and suggested the Beacon variety as a parent in breeding for high vitamin C content. In 1947 Darrow *et al.* (4), and Ezell *et al.* (5), concluded that it is quite possible to breed varieties for either higher or lower vitamin C than any now available.

MATERIALS AND METHODS

The strawberry populations used for this study were from crosses made in 1946. The object of the original experiment (10) was to test the combining-ability, with respect to vegetative vigour and resistance to leaf spot, of clones from several inbred strawberry lines originating from different varieties. Nine clones were chosen with regard to their leaf-spot reaction. Each of these was selfed and crossed with all others. Their progenies were planted in 2 complete randomized blocks. The plants were spaced 4 feet 6 inches on the square and allowed to produce runners without restriction. The parents were planted in an adjacent block and, while under similar conditions, were not strictly comparable with their progeny.

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The first fruit from the parental clones was harvested in June, 1948, and analysed for vitamin C content. Of the 9 original parents, 5 were chosen for study on the basis of their vitamin C content. No selection had been made previously for vitamin C content in any of these lines. The breeding numbers, source, and number of generations selfed were as follows: 3819–22, Marshall, 3 generations selfed; 3632–2, Dunlap, 3 generations selfed; 33142–9, Minnehaha, 2 generations selfed; 3315–7, Chaska, 2 generations selfed; and 382–89, Howard 17 (Premier), hereafter referred to as Premier, 1 generation selfed. These parents will be called Marshall 3, Dunlap 3, Minnehaha 2, Chaska 2, and Premier 1 in the remainder of the paper. The direction in which the crosses were made and the sizes of the populations studied are shown in Table 1.

Table 1.—List of selfs and crosses, and the size of populations studied

Parentage	Number of Plants	Parentage	Number of Plants
Marshall 3 × self	16	Chaska 1 × self	19
× Dunlap 3	22	× Marshall 3	35
× Minnehaha 2	29	× Dunlap 3	36
Dunlap 3 × self	12	× Minnehaha 2	25
× Marshall 3	4	× Premier 1	23
Minnehaha 2 × self	22	Premier 1 × self	31
× Dunlap 3	41	× Marshall 3	35
× Premier 1	32	× Dunlap 3	30
Total			412

Fully ripe, undamaged strawberries were harvested for vitamin C analyses from June 13 to June 19. Up to 50 gm. of berries, with calyx attached, were harvested from individual plants and immediately placed in numbered cellophane bags. The bags were heat sealed, making them air-tight, and placed in a freezer at −10° F. within 3 hours from the time of harvest. The samples were stored at −10° F. until analysed. Further samples from the parent plants were taken between the above dates.

Samples of 5 standard varieties were harvested from the University of Minnesota Fruit Breeding Farm, Excelsior, or the Mayo Forestry and Horticultural Research Institute, Rochester, or from both. From 3 to 6 independent determinations were made on each sample and the results compared with those reported in the literature.

In order to estimate the amount of vitamin C lost from the berries under the conditions of storage used, samples of the Premier variety in a yield trial were taken from each of 3 replicates. Each sample was divided into 10 sub-samples and packaged in individual cellophane bags. At the beginning, end, and three times during the period of analysis, 2 samples from each of the 3 replicates were drawn at random and analysed.

The vitamin C analyses were made according to Ballentine (1). Samples of about 30 gm. (from 15 gm. to 35 gm.) of the frozen fruit were hulled, counted and weighed to 0.1 gm. They were mascerated with about 30 ml. of 4 per cent metaphosphoric acid in a semi-micro Waring Blendor. The resulting slurry was quantitatively transferred to a 100 ml. graduate and brought up to volume with metaphosphoric acid. Where less than 25 gm. of fruit were available the final volume was adjusted accordingly. The slurry was immediately filtered and the vitamin C content of the filtrate determined by the potassium iodate method on at least two 10 ml. aliquots.

The statistical analysis of the data was made according to Snedecor (13). The analyses of the plot means made use of the usual analysis of variance adjusted for the unequal numbers of plants per plot by the general method of fitting constants.

RESULTS

Comparison with Values Previously Reported

The means of the vitamin C determinations of 6 samples from each of 5 varieties are shown in Table 2 (Column 1E and 1R). The vitamin C content of these varieties, as reported in the literature, is listed for comparison.

TABLE 2.—A COMPARISON OF VITAMIN C DETERMINATIONS OF STANDARD VARIETIES WITH THOSE REPORTED IN THE LITERATURE

Variety	Mean vitamin C content in mgm. per 100 gm. as reported by*:					
	1E	1R	2.	3.	4.	5.
Aberdeen	—	40.1	40.9	50	41	—
Dunlap	103.8	—	72.2	—	—	79
Marshall	81.5	78.7	67.4	—	49	—
Midland	—	68.9	60.8	86	64	—
Premier	78.0	58.7	66.6	63	54	—

* 1E — Fruit from Excelsior
1R — Fruit from Rochester
2. — Esell, Darrow, Wilcox, and Scott (5)
3. — Darrow, Wilcox, Scott, and Hutchins (4)
4. — Slate and Robinson (12)
5. — Mayfield and Richardson (8)

While it is not possible to compare these results critically, it will be noted that those samples harvested from Rochester had vitamin C contents closely approaching those reported in the literature. The 3 standard varieties harvested from Excelsior all gave vitamin C contents which appear to be higher than the results previously reported. This discrepancy might be explained by the fact that during the period of harvest light intensity was high at Excelsior. There was also a lack of moisture during the ripening period which may have reduced the water content of the fruit.

Loss of Vitamin C While in Storage During the Period of Analysis

The means of 6 determinations for each of 5 dates on the Premier variety harvested from Rochester are shown in Table 3.

TABLE 3.—VITAMIN C CONTENT OF CONTROL DETERMINATIONS FOR DATE OF ANALYSIS. (PREMIER VARIETY FROM ROCHESTER, MINN.)

Date of Analysis		Vitamin C content (Mean of 6 determinations)
		mgm. per 100 gm.
July	15	57.6
	21	59.7
	28	57.7
August	7	59.6
	18	58.5

The analysis of variance, using individual samples rather than the means of two samples (a total of 29 degrees of freedom rather than 14 degrees of freedom) gave an *F* value less than expectation for "dates" source and an *F* value of 7.6 for "replicates" source of variation. There were, therefore, no differences among the determinations of the Premier variety during the month of analysis under the conditions of this experiment and the first and last determinations were directly comparable.

Vitamin C Content of the Parents, the Self, and the Cross Populations

The mean vitamin C content of the parental clones, the self, and the cross populations from these parents are presented in Table 4. The parents used in this study fell into 2 distinct categories with respect to the vitamin C

TABLE 4.—MEAN VITAMIN C CONTENT OF SELF AND CROSS POPULATIONS IN MGM. PER 100 GM.

Parents	Marshall 3	Dunlap 3	Minnehaha 2	Chaska 2	Premier 1
Mean of parental Clones	126.0±3.3	116.5±4.5	109.7±4.2	85.3±3.1	79.6±3.8
Premier 1	129.2	104.6	108.8	96.7	102.1*
Chaska 2	118.3	106.3	113.8	104.6	
Minnehaha 2	134.9	115.7	110.7		
Dunlap 3	131.3	117.2			
Marshall 3	128.7				
Mean of means of crosses	128.4	114.5	118.3	108.8	109.8

* Means of selfed populations are underlined

	Between Means of:	
L.S.D. at	Crosses and Selfs (Body of Table)	Means of Crosses (Foot of Table)
5 per cent	11.2	7.2
1 per cent	15.6	9.8

content of their fruit. Marshall 3, Dunlap 3, and Minnehaha 2 were considered to have very high vitamin C contents while Chaska 2 and Premier 1 were medium in their vitamin C content. These two groups were significantly different. Differences within these groups were not demonstrated.

The vitamin C content of the strawberry populations studied was highly variable. The extent of this variability is shown in Table 5, which presents the frequency distribution in percentage of the plants with vitamin C contents in the classes indicated. The crosses tended to be more uniform than the selfs with respect to the vitamin C content of their fruit, but not significantly so. The variability of the strawberry populations with respect to vitamin C content was due to the variability of samples from the same plant, the variability of a chemical determination, and actual differences among plants within a given family. The actual differences among plants within a family may be attributed to genetical and environmental differences.

It was estimated by use of the true variance that the environmental variation between plots of the same cross family was 2.1 per cent of the total variation; the environmental variation between plots of the same self family was less than expectation. Therefore much of the within-family variation may be attributable to genetic differences between plants within a family. The estimate of the true variance showed that 67.4 per cent

TABLE 5.—FREQUENCY DISTRIBUTION IN PERCENTAGE OF POPULATION TOTALS. VITAMIN C CONTENT OF STRAWBERRY FRUIT. (MGM. PER 100 GM.)

Population	Class Centres (mgm. per 100 gm.)										
	65	75	85	95	105	115	125	135	145	155	165
Marshall 3 selfed			6		19	13	19	13	12	12	6
× Dunlap 3					12	15	19	19	27		8
× Minnehaha 2				4		10	24	31	17	4	10
× Chaska 2			6	6	14	20	34	17		3	
× Premier 1				9	14	14	17	9	20	9	8
Dunlap 3 selfed	8	8		8		9	42		25		
× Marshall 3					12	15	19	19	27		8
× Minnehaha 2			5	7	24	34	13	10	3	2	2
× Chaska 2		8	14	17	25	9	19	8			
× Premier 1			27	13	23	13	20	4			
Minnehaha 2 selfed	6		14	9	17	29	20	3	2		
× Marshall 3				4		10	24	31	17	4	10
× Dunlap 3			5	7	24	34	13	10	3	2	2
× Chaska 2		4	4	16	32	12	12	12	4		4
× Premier 1		6		19	31	19	19	6			
Chaska 2 selfed			16	26	21	26	11				
× Marshall 3			6	6	14	20	34	17		3	
× Dunlap 3		8	14	17	25	9	19	8			
× Minnehaha 2		4	4	16	32	12	12	12	4		4
× Premier 1	4	5	26	22	26	13	4				
Premier 1 selfed		7	16	29	19	16	7	3	3		
× Marshall 3				9	14	14	17	9	20	9	8
× Dunlap 3			27	13	23	13	20	4			
× Minnehaha 2		6		19	31	19	19	6			
× Chaska 2	4	5	26	22	26	13	4				

of the total variability was found between plants within a family compared with only 30.5 per cent found between families of selfs and families having one parent in common. The conclusions to be drawn from these estimates indicate that the parents were highly heterozygous for vitamin C content. However, the parents had undergone no selections for vitamin C content in the course of the breeding work.

A consideration of the mean vitamin C contents of the various self and cross families presented in Table 4 shows that there were significant differences among families. There was a close association ($r = .75$, P less than .01) between the mean of a cross population and the mean of the 2 parents involved in the cross. There was a similar association ($r = .80$, P less than .01) between the mean of a cross and half the sum of the means of the selfed populations derived from the parents involved in the cross. In no case were the means of the crosses significantly greater than the means of the higher parents, although in several cases they were equal to the higher parent. It may be concluded that high vitamin C content of strawberry fruit is partially dominant to low vitamin C content with the parental material used. There was no evidence of heterosis for this character. Marshall 3 appeared to transmit its high vitamin C content particularly well, especially in combination with Minnehaha 2, Dunlap 3, and Premier 1, while Minnehaha 2 transmitted its high vitamin C content well in all crosses except with Premier 1.

Since a vigour rating had already been made on these plants, and since the weight per fruit of the samples used for analyses was available, simple correlations between these three characteristics were made. The results of these correlations are presented in Table 6. It is of some interest to note the significant, but low, negative correlation between weight per fruit and vitamin C content. This had previously been reported within varieties and apparently holds true among varieties. The large fruit tended to contain less vitamin C per unit weight than did the small fruit. The correlation between plant vigour and weight per fruit was low, but significantly positive as would be expected.

TABLE 6.—CORRELATIONS (r) BETWEEN VITAMIN C CONTENT, WEIGHT PER FRUIT, AND PLANT VIGOUR

Character:	Vitamin C Content			Weight per fruit		
Population:	Selfs	Crosses	Total	Selfs	Crosses	Total
Vigour	−.140	−.078	−.032	+.027	+.189**	+.292**
Weight per Fruit	−.077	−.182**	−.103*			

* Significant at 5 per cent
** Significant at 1 per cent

DISCUSSION

The results of these studies indicate that not only is high vitamin C partially dominant to low vitamin C but the parents, when selfed, produce some plants having a higher vitamin C content than their own. The crossed populations also contain some plants having a higher vitamin C content than do the parents, and in some cases higher than any found in the selfed populations from their parents. This indicates a recombination of favourable genes within a parent or from both parents. Therefore the development of a strawberry variety containing more vitamin C than any variety now available could probably be accomplished without difficulty. The fact that there is a negative, but low, correlation between fruit size and vitamin C content should not seriously hinder such a program. The appearance of large fruited, high vitamin C individuals may occur frequently enough to allow for the selection of other desirable characteristics within this group of individuals. The matter of vigour needs little consideration if inbred clones are used as parents, because it has been shown that all crosses with these particular inbred selections, at least, produce progeny with satisfactory vigour.

The advantages to be gained by a study of the selfed progeny from the parental material are shown in the added information regarding the genotypes of these parents. Another advantage from selfing, not demonstrated in this study, is the selection of desirable lines within these selfed populations. The within-family variance measures the variability of the selfed families. Such a statistic, applied to selfed families produced from plants with families of known variance, should be of greater use in assessing the uniformity of any such selected line. It would then be possible not only to study the direction in which the mean was being selected but also to measure the change in variability brought about by such selection. It is quite conceivable that if the individuals within the selfed families here reported were selfed, some would produce families having equal, and some having less, variability than the line from which they were derived. This information, together with the means of the characteristic being studied would be of use in assessing their respective breeding values.

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DDT RESIDUES ON CELERY RESULTING FROM DUST TREATMENTS FOR CONTROL OF THE TARNISHED PLANT BUG[1]

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INTRODUCTION

In Manitoba, "black joint" of celery, attributed to the feeding activity of the tarnished plant bug, *Lygus oblineatus* (Say), has caused considerable concern. DDT was shown in 1944 to be very effective against the nymphs and adults of this insect (1, 4). Satisfactory control has been obtained with 3 to 5 per cent DDT dusts (2, 3). Because DDT dusts are being used by celery-growers in Manitoba to control this insect, it is desirable to know the amount of DDT residues present at harvest-time and when the celery is marketed.

Applications of DDT dust were made to celery crops throughout the growing season, and the deposits of DDT remaining on the leaf-stalks at harvest time were determined. It appeared possible that DDT deposits might accumulate differentially within the celery heads, especially because overhead irrigation was being used. Accordingly, the distribution of the residues at different locations on the celery plants was measured separately. The reduction of residues caused by post-harvest trimming and commercial washing prior to marketing was also investigated.

APPLICATION OF INSECTICIDE

The dusts were applied independently by a market-gardener using field machinery. All dusting was carried out during the evening. The outlets of the dusting machine were set about 6 inches above the celery plants, so that the dust would be driven well into the foliage and heads.

The applications were as follows:—

(1) In 1947 a 3 per cent DDT dust was applied at 35 lb. per acre on July 15 and August 1, 14, and 23, making a seasonal total of 4.2 lb. of actual DDT per acre.

(2) In 1948, early-harvested celery received a 5 per cent dust at 50 lb. per acre on July 8, and a 3 per cent dust at 75, 35, and 35 lb. per acre on July 20 and August 5 and 14, respectively, making a seasonal total of 6.8 lb. of DDT per acre.

(3) In 1948, late-harvested celery received the treatments applied to early-harvested celery and 3 additional applications of 3 per cent dust at 35 lb. per acre on August 21 and 24 and September 3, making a seasonal total of 10.0 lb. of DDT per acre.

SAMPLING, AND ANALYSIS OF RESIDUES

In September, samples of celery were taken for residue analysis. In 1947, samples were taken 13 days after the last dust treatment; in 1948, 25 days and 4 days elapsed between the last dusting and the sampling of the early-harvested and the late-harvested celery, respectively. The series of analyses to determine the reduction of residues resulting from post-harvest processing was made on late-harvested celery 20 days after dust treatments had been completed.

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In 1947, leaf-stalk samples were taken from 18 treated plants in the following manner. A celery-bed was selected and 2 outside leaf-stalks were removed from every third plant in a row. This whole sample was then divided into 3 sub-samples, each containing 12 leaf-stalks. Each leaf-stalk was divided into 2 "stalk zones", which have been termed "upper" and "basal". The upper zone comprised the portion of the leaf-stalk from the first node down to the top of the membranous structure that runs along the edge of each leaf-stalk in the basal region and serves to clasp firmly the inner stalks. The basal zone extended from this point to the top of the root. Thus the distribution of residues at 2 locations on the outside leaf-stalks of celery heads was measured. A control sample was prepared by taking 6 outside leaf-stalks from each of 2 whole plants that had not received treatment.

In 1948, 10 whole plants were selected at random from each of the treated beds. Each plant was arbitrarily divided into 3 stalk locations:—

(1) *Outermost*—the outer broad leaf-stalks that firmly surround the celery head;
(2) *Middle* —leaf-stalks midway between the outermost leaf-stalks and the heart;
(3) *Heart* —the first recognizable leaf-stalks next to the unfolded heart.

Two leaf-stalks were taken from each of these locations. The leaf-stalks were in turn divided, as in 1947, into 2 stalk zones. Thus the distribution of residues was determined at 6 places within the celery heads. Samples from each of the above-mentioned stalk locations and zones were composited for the 10 plants. Control samples were prepared in the same manner.

Preliminary steps in sample preparation consisted of removing the old, decumbent leaf-stalks surrounding the celery heads, cutting off the roots, and washing off any adhering soil. After the composite samples were prepared, they were weighed, and the DDT residues were removed with reagent grade benzene.

The reduction of residues on celery resulting from commercial washing was determined as follows: Sixteen days after the residues were measured on late-harvested celery, 20 plants that had been trimmed for market were obtained from crates in the warehouse. Half of these plants were washed with a "bunch washer", and then both lots were sampled by the system outlined. The bunch washer consisted of a travelling belt which carried the celery heads between two groups of 11 spray nozzles, one directed from above the moving celery, the other from below. Water emerged from the nozzles under a pressure of 300 lb. per square inch.

In 1947, the DDT residues from the upper and basal stalk zones were stripped into a large funnel, fitted with a tight plug of glass wool, and placed over a 200-ml. volumetric flask. While each portion of the stalk was slowly revolved over the funnel, a stream of benzene dispensed from a wash-bottle was directed up and down the sides several times. After approximately 190 ml. of strippings were thus collected, 10 ml. of benzene were used to wash down the funnel.

In 1948, each composite sample, consisting of 150 to 200 gm. of celery, was placed in a glass-stoppered reagent jar and washed successively with 90, 45, and 45 ml. of benzene. The celery and benzene were shaken together for 2 minutes during each washing. The jar was emptied and rinsed with 20 ml. of benzene. The washings from each sample were filtered through glass wool into a 200-ml. volumetric flask.

To check the efficiency of the residue extraction method, 2 samples of celery from which the DDT residues had previously been extracted were re-washed with 50 ml. of benzene each and DDT analyses made. The results were negative for both samples.

Before determination, 2 gm. of anhydrous sodium sulphate were added to each 200 ml. of residue extract to dehydrate the sample and hasten clarification. The Schechter-Haller (6) spectrophotometric method was used for the DDT analyses. Results from the control samples were used to correct the values observed for treated celery.

RESULTS AND DISCUSSION

Several applications of DDT dust during the growing season produced high residues on celery, particularly at the base of the head, toward the periphery. As shown in Table 1, increased DDT residues on celery resulted when the total amount of DDT applied was increased. After more than 4.2 lb. of DDT per acre were applied in a season, residues exceeding the tentative tolerance level of 7.0 p.p.m. were found in the basal zones of the outermost and middle leaf-stalks.

TABLE 1.—DISTRIBUTION OF DDT RESIDUES ON CELERY PLANTS

Total DDT pounds per acre	Days last application to harvest	Stalk zone	Residues		
			Stalk location		
			Outermost (p.p.m.)	Middle (p.p.m.)	Heart (p.p.m.)
4.2[1]	13	Upper	1.37	—	—
		Basal	2.77	—	—
6.8[2]	25	Upper	2.20	0.85	0.45
		Basal	24.10	7.90	0.84
10.0[3]	4	Upper	4.60	2.23	2.20
		Basal	32.90	26.05	3.10

[1] 1947. Average of 3 sub-samples.
[2] 1948. Early-harvested celery.
[3] 1948. Late-harvested celery.

The application of 6.8 lb. of DDT per acre during the season resulted in a residue of 24.1 p.p.m. at the bases of the outermost leaf-stalks, 25 days after the last dust application. After 3 additional applications of 3 per cent dust at 35 lb. per acre, the residue, measured 4 days after the last application, increased in this location to 32.9 p.p.m. At the bases of the middle leaf-stalks the corresponding residue increased from 7.9 to 26.0 p.p.m. In all cases, residual deposits were low on the upper zone at each stalk location. Residues were especially low on the leaf-stalks close to the heart.

It was expected that post-harvest processing would effect a significant reduction of residues, because the leaf-stalks designated as outermost were removed and the remainder of the head was washed before marketing. Accordingly, the middle and heart locations from the 2 lots of celery were sampled as before, as well as outer leaf-stalks that remained after removal of the outermost stalks. The results are shown in Table 2.

TABLE 2.—REDUCTION OF DDT RESIDUES ON TRIMMED LATE-HARVESTED CELERY EFFECTED BY WASHING

Treatment	Stalk zone	Residues		
		Stalk location		
		Outer (p.p.m.)	Middle (p.p.m.)	Heart (p.p.m.)
Unwashed	Upper	2.22	1.05	0.85
	Basal	11.72	4.40	1.32
Washed	Upper	1.12	0.61	0.58
	Basal	4.5	2.02	1.20
Per cent reduction	Upper	49.6	42.0	31.8
	Basal	61.6	54.0	9.1

In the 16-day interval between analyses, the residues at the middle and the heart locations, after a total seasonal dosage of 10.0 lb. of DDT per acre, dropped markedly (Table 2). It may be assumed that removal of the outermost leaf-stalks resulted in a significant reduction of the total residue. In addition, commercial washing removed from 40 to 60 per cent of the residue remaining after harvest.

Manalo *et al.* (5) determined DDT residues on celery after application of a 5 per cent DDT dust. The celery was divided into mature stalks and leaves and young stalks and leaves. The latter sample, after weighing, was washed with water to remove dirt, in a manner simulating preparation for the table. They found DDT deposits of 13.16 p.p.m. on the unwashed mature stalks and leaves 3 days after application and only 1.62 p.p.m. 18 days later. Young stalks and leaves that were washed before analysis retained DDT deposits of 0.5 and 1.22 p.p.m., respectively, after the same intervals.

In the present study, DDT residues were found to be differentially distributed throughout celery heads after frequent and heavy applications of dusts. The average total deposits increased progressively with rates of application. The residues were excessively high at the base of the head, especially toward the periphery. A reduction from 26.05 to 4.4 p.p.m. at the base of the middle stalk after 16 days' weathering was similar to that reported by Manalo *et al.* (5). Reduction during this period was less in other stalk locations. Appreciable amounts of DDT were removed with the outermost leaf-stalks at harvest, and commercial washing effected a further reduction.

SUMMARY

1. The distribution of DDT residues on celery plants treated with DDT dust at 4.2, 6.8, and 10.0 lb. of actual DDT per acre during the season was determined.

2. The DDT residues increased with rate of application and were found to be greater at the bases of the leaf-stalks than at the distal ends; deposits increased peripherally from the heart.

3. Application of 6.8 lb. of DDT per acre resulted in a deposit of 24.1 p.p.m. at the bases of the outermost leaf-stalks; when the seasonal application reached 10.0 lb., the residue in this location increased to 32.9 p.p.m. At the bases of the middle leaf-stalks the corresponding increase was from 7.9 to 26.0 p.p.m. In all cases, residual deposits were low on the upper zone at each stalk location. Residues on the leaf-stalks close to the heart were especially low.

4. Removal of the outermost leaves at harvest appears to produce a large reduction in the total DDT residue. Commercial washing accounted for a reduction of approximately 40 to 60 per cent of the remaining residue. The highest residual deposit left on the celery plants after washing was 4.5 p.p.m.

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DDT RESIDUES ON CURRANTS AND GOOSEBERRIES[1]

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INTRODUCTION

Experiments on the control of the currant fruit fly, *Epochra canadensis* Loew, have been carried out in Manitoba for several years. As a result of these experiments, DDT has been recommended for the control of the fly (1).

To determine the rates and number of applications required to obtain residues below the tentative tolerance level of 7.0 p.p.m., assessments of DDT residues were made on the treated fruit at harvest. Procedures for sampling the fruit, and extracting and measuring the residues, were investigated in 1946 and 1947. The information obtained suggested that DDT sprays applied after flowering, at high rates per acre, would probably result in excessively high residues on the ripe fruit. Accordingly, in order to build up effective DDT residues on the stems and foliage of the plants without increasing the deposition on the fruit, pre-bloom sprays were applied to the currants in the bud stage. In addition, post-bloom sprays were employed to increase and extend the usefulness of the DDT coverage. The first post-bloom spray was applied at the cessation of bloom; the second, when applied, followed 10 days later. The DDT residues on the ripe fruit resulting from different rates and combinations of pre- and post-bloom sprays were determined in 1948 and 1949. The interval from the last spray application to harvest and the precipitation during this period were considered.

METHODS

Field Plots and Arrangement

In 1948 the plots, which were located on several farms, each consisted of 10 to 20 currant or gooseberry bushes. Two treatments were used; each was applied to 4 plots, irrespective of the type of fruit. In 1949 each of the plots consisted of 14 red currant bushes. Six plots were arranged in each of 3 adjacent blocks. Six treatments were randomized within each block.

A 3-gal. compressed air sprayer was used to apply 50 per cent wettable DDT powder.

Sampling and Analysis

Fruit samples for residue analysis were taken when the fruit was ripe. A sample consisted of berries picked at random from one plot. The samples were quick-frozen shortly after collection and stored in a frozen state until the extractions were made.

In 1948, each 226-gm. sample was placed in a 1000-ml. Squibb separatory funnel and washed 3 times, once with approximately 95 ml. and then twice with 50-ml. portions of reagent grade benzene. The funnel was shaken continuously for 2 minutes during each washing. After the sample

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TABLE 1.—DDT RESIDUES IN PARTS PER MILLION ON RED CURRANTS (R) AND GOOSEBERRIES (G), 1948

Treatment		Fruit	Rainfall first spray to harvest inches	Days last spray to harvest	Residues
Number of sprays	Total DDT pounds per acre				
		R	4.87	47	1.63
1 pre-bloom	2.3	R	4.97	49	5.30
1 post-bloom	1.15	R	4.97	49	2.50
		G	4.97	48	1.85
					Average 2.82
		R	9.07	36	3.34
2 post-bloom	2.3	R	4.97	37	2.67
		G	9.07	36	2.37
		G	4.97	37	2.90
					Average 2.82

was discarded the funnel was washed with approximately 10 ml. of benzene. The benzene extracts were filtered through glass wool to remove benzene-insoluble matter and were collected in a 200-ml. volumetric flask. Two grams of anhydrous sodium sulphate were then added to the flasks to hasten clarification of the residue extracts. Duplicate aliquots were taken and the DDT content was measured by the Schechter-Haller spectrophotometric method (2).

To determine the efficiency of the residue-stripping procedure, check tests were made on several samples. One additional wash with 50 ml. of benzene was given these samples, and only 0.0 to 0.05 p.p.m. was recovered after this extraction.

In 1949, 350-gm. samples were used. They were placed in a 1000-ml. Squibb separatory funnel and extracted successively with 125, 75, 75, and 50 ml. of reagent grade benzene; stripping was effected by shaking for 5, 3, 2, and 2 minutes, respectively. After a sample was discarded the separatory funnel, the filter funnel, and the glass wool were washed with 3 successive 15-ml. portions of benzene. The final volume of the total extract was adjusted to 350 ml., and 5 gm. of anhydrous sodium sulphate were added. Aliquots were taken and analysed for DDT as in 1948.

RESULTS AND DISCUSSION

The basic spray rate used in pre- and post-bloom sprays was 1.15 lb. of actual DDT per acre, which is approximately equivalent to an application of 0.5 lb. of DDT in 100 gal. of water, applied until the foliage drips. This rate has been recommended for the control of the currant fruit fly (1).

In 1948, 2 treatments were used, as shown in Table 1. The first consisted of 1 pre-bloom application of 2.3 lb. of DDT per acre, followed at cessation of bloom by an application of 1.15 lb. of DDT per acre. The second treatment consisted of 2 post-bloom sprays applied at 1.15 lb. of DDT per acre, 10 days apart.

As Table 1 shows, the DDT residues measured were below the tentative tolerance level of 7.0 p.p.m. No significant differences were established between the treatments.

In 1949, 6 treatments were employed. The pre-bloom rates were 0.0, 2.3, and 4.6 lb. of DDT per acre, and 2 post-bloom sprays were applied 10 days apart, one at the basic rate of 1.15 lb. of DDT per acre, as in 1948, and the other at double that rate, as shown in Table 2.

TABLE 2.—DDT RESIDUES IN PARTS PER MILLION ON RED CURRANTS 39 DAYS AFTER THE LAST SPRAY APPLICATION, 1949

Treatment Total DDT pounds per acre Pre-bloom 1 spray	Treatment Total DDT pounds per acre Post-bloom 2 sprays	Rainfall first spray to harvest inches	Residues Maximum	Residues Average[1]
0.0	2.3	4.54	3.53	2.71
2.3	2.3	6.38	4.07	3.01
4.6	2.3	6.38	5.22	4.22
0.0	4.6	4.54	7.42	6.11
2.3	4.6	6.38	5.46	5.22
4.6	4.6	6.38	7.42	6.21

[1] Difference required for significance at 5 per cent level: 1.88.

A comparison of Tables 1 and 2 shows that in both years 2 post-bloom applications at the basic rate resulted in residues approximating 3.0 p.p.m. In 1949, the pre-bloom sprays which preceded the 2 post-bloom sprays at the basic rate appear to have affected the residual deposits, but these differences are not significant. However, when the post-bloom spray rate was increased to 2.3 lb. of DDT per acre, residues were significantly increased. The averages fall into 2 groups corresponding to the post-bloom spray rates, and a significant difference in residues was demonstrated between these post-bloom spray rates.

The results obtained in both years show that pre-bloom sprays, as would be expected, have little influence on the residue at harvest. One post-bloom application of 1.15 lb. of DDT per acre gave rise to moderate residues. Residues resulting from 2 post-bloom sprays at the same rate were also moderate. But when 2 post-bloom sprays at double the basic rate, or 2.3 lb. of DDT per acre, were applied, residues approximated the tentative tolerance level of 7.0 p.p.m.

There was no evident relationship between the DDT residues on the harvested fruit and the period from the last spray application to harvest or the precipitation during this interval.

SUMMARY

1. In experiments designed to control the currant fruit fly, DDT residues were determined on harvested red currants and on gooseberries that had been treated with different rates and combinations of pre- and post-bloom sprays.

2. Pre-bloom sprays do not apparently influence residues at harvest. No significant difference was found between rates of 0.0, 2.3, and 4.6 lb. of DDT per acre.

3. The DDT residues after 1 post-bloom spray of 1.15 lb. of DDT per acre, which is equivalent to a thorough application of 0.5 lb. of DDT in 100 gal. of water, approximated 3.0 p.p.m. Two post-bloom sprays at the same rate, which have been recommended for control of the currant fruit fly, resulted in similar residues.

4. Residues were significantly increased and approached the tentative tolerance level of 7.0 p.p.m. when 2 post-bloom sprays were applied at 2.3 lb. of DDT per acre.

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FEEDING VALUE OF HORSEBEAN (*Vicia faba* L.) FOR CHICKS[1]

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An analysis of Canadian crop production reveals an inadequate volume of crops yielding vegetable protein of suitable biological quality for poultry feeding. Climatic adaptability is a foremost prerequisite in any crop whose production could be encouraged to meet this deficiency. The horsebean (*Vicia faba* L.) has already been grown to a limited extent in Quebec and the Maritime Provinces, and would appear to offer some promise in this development in these areas. It is a leguminous plant which is well adapted to a cool humid climate, the seed possesses fairly high protein content (25–27 per cent), and there is a good yield on any reasonably fertile soil.

McKee (20) and Bassot (4) stated that the horsebean possesses a high nutritive value for horses, cattle, swine and sheep. Belzile (5), however, concluded that horsebean was not superior to alfalfa, red clover or the field pea when fed as green forage. Woodman and Evans (25) reported that horsebean-pod meal had a very satisfactory digestibility, roughly two-thirds of the nutrients being available to ruminants. Paquette (21) studied, in vitro, the enzymatic digestibility of raw *Vicia faba* protein by pepsin and trypsin. He found that horsebean protein resembled soybean protein more closely than casein with respect to its digestibility. Zagami (26) reported that *Vicia faba* was adequate in vitamins B (B_1 by more recent classification) and E as shown by normal reproduction and absence of beriberi in rats.

Although the horsebean has demonstrated fairly high feeding value for larger animals, no experiments are reported in the literature on its nutritive value for poultry. Investigations were, therefore, undertaken to study the comparative feeding value of horsebean seed meal in chick rations.

EXPERIMENTAL

As a preface to experimental studies on the feeding value of the horsebean for chicks, it was necessary to have pertinent chemical data. Chemical analyses were carried out on two types of horsebean, small- and large-seeded varieties. A summary of chemical analyses is presented in Table 1.

The general pattern of all the chick experiments was the same for all trials. Male Barred Plymouth Rock chicks were started on a commercial ration for one week after which they were randomized into lots, wing-banded and confined to electrically heated wire floored battery brooders. The chicks were fed ad libitum throughout and had access to fresh water continuously. Body weight and feed consumption data were recorded each week. The basal horsebean meal ration for all trials is given in Table 2.

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TABLE 1.—COMPOSITION OF HORSEBEAN SEED

Component Dry Matter Basis	Large Seeded-Type Whole Seed	Small Seeded-Type Whole Seed
	%	%
Moisture	12.6	9.8
Crude Fat	1.6	1.7
Crude Protein	27.2	26.7
Crude Fibre	9.2	9.2
Ash	3.8	4.0
N-Free Extract	55.6	58.3
Calcium	0.14	0.15
Phosphorus	0.51	0.33
Thiamin (1)	225	220
Riboflavin (1)	175	210

[1] Gamma per 100 grams of dry matter.

TABLE 2.—COMPOSITION OF THE BASAL HORSEBEAN SEED MEAL RATION

Ingredients	Pounds
Hominy Feed	20
Ground Wheat	15
Ground Barley	10
Wheat Middlings	10
Alfalfa Meal (Dehyd.)	5
Brewers' Yeast	2
Ground Limestone	2
Salt (Iodized)	1
Horsebean Seed Meal	35
Fish Oil (1850 A, 400 D_3)	100 gm.
Riboflavin (Synthetic)	0.08 gm.
$MnSO_4 . 4H_2O$	6.0 gm.
Total[1]	100

[1] Calculated per cent Crude Protein........................18.5

Preliminary observations indicated that normal feather colour in Barred Plymouth Rock chicks was disturbed by high levels of horsebean meal feeding. In order to facilitate the scoring of this abnormal feathering, the chicks were fed a commercial ration for two weeks at the end of the experimental period. They were then examined with special attention to earlier feather development and pigmentation. The latter were scored separately on a basis where a rating of five indicated very poor feather development or total absence of black pigment while excellent feather growth and uniform barring were scored zero. To ensure unbiased scoring, chicks from all lots were mixed together and then drawn at random.

RESULTS AND DISCUSSION

Experiment 1

It is known that plant protein supplements cannot be fed as the sole source of protein in chick rations if maximum growth is desired. Vegetable proteins must be supplemented with varying amounts of animal protein to

TABLE 3.—COMPOSITION OF THE RATIONS FED IN EXPERIMENT I

Ingredient	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6	Lot 7
Basal Mixture	65.0	65.0	65.0	65.0	65.0	65.0	65.0
Hominy Feed	—	5.6	7.7	7.7	9.4	11.8	13.3
Raw Horsebean	35.0	17.5	17.5	17.5	17.5	17.5	17.5
Linseed Oil Meal[1]	—	11.9	—	—	—	—	—
Soybean Oil Meal	—	—	9.8	—	—	—	—
Sunflower Seed Oil Meal	—	—	—	9.8	—	—	—
Meat Meal	—	—	—	—	8.1	—	—
Fish Meal	—	—	—	—	—	5.7	—
Casein	—	—	—	—	—	—	4.2
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated per cent Crude Protein	18.5	18.7	18.6	18.5	18.5	18.5	18.6

[1] Water Treated.

TABLE 4.—COMPLEMENTARY VALUE OF SOME PLANT AND ANIMAL PROTEIN SUPPLEMENTS IN A BASAL HORSEBEAN SEED MEAL RATION

Lot No.	Supplement Replacing Raw Horsebean Seed Meal	Final Weight at 6 Weeks[1]	Feed: Gain Ratio	Feathering	
				Growth Score	Achroma Score
		Grams			
1	Raw Horsebean Alone	160.2	4.11	3.4	3.9
2	Linseed Oil Meal[2]	219.5	3.44	1.7	0.8
3	Soybean Oil Meal	246.9	3.40	2.0	3.4
4	Sunflower Seed Oil Meal	324.4	3.11	0.9	1.3
5	Meat Meal	271.6	3.30	2.2	1.7
6	Fish Meal	349.0[3]	2.56	0.7	0.0
7	Casein	306.9	3.04	0.5	1.5

[1] Greatest necessary difference for significance of final weight is 51.0 at the 0.05 level.
[2] Water treated.
[3] Average of 4 chicks only due to accidental mortality.

induce best growth. Animal proteins, however, differ greatly in their ability to supplement plant proteins. Likewise, vegetable protein supplements exhibit different complementary values with each other in practical chick rations (9, 14, 15, 24).

The purpose of this initial experiment was to investigate the replacement value of some plant and animal protein supplements in the basal horsebean meal ration.

On the basis of chemical analyses, one-half of the horsebean seed meal protein was replaced in the basal ration (Table 2) by the following protein supplements: Linseed oil meal (water treated (17)), soybean oil meal, sunflower seed oil meal, meat meal, casein, and fish meal. Because of differences in protein content of the supplements added, adjustments were made at the expense of hominy feed (Table 3). Fourteen chicks per lot

TABLE 5.—COMPOSITION OF THE RATIONS FED IN EXPERIMENT 2

Ingredient	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6	Lot 7	Lot 8	Lot 9
Animal Protein per cent of Total Crude Protein	0.0	10.0	20.0	10.0	20.0	10.0	20.0	—	—
Basal Mixture	45.0	45.0	45.0	45.0	45.0	45.0	45.0	45.0	45.0
Horsebean Seed Meal	25.2	25.2	25.2	25.2	25.2	25.2	25.2	25.2	25.2
Hominy Feed	20.8	21.9	22.5	22.5	23.6	23.0	24.3	20.2	19.8
Soybean Oil Meal	9.0	4.0	—	4.0	—	4.0	—	7.5	6.0
Meat Meal	—	3.9	7.3	1.9	3.6	—	—	—	—
Fish Meal	—	—	—	1.4	2.6	3.0	5.5	—	—
Condensed Fish Solubles	—	—	—	—	—	—	—	2.0	4.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated per cent Crude Protein	19.1	18.9	19.0	18.9	19.0	19.1	19.1	19.0	19.0

were fed on the experimental diets for a period of five weeks. The results of this experiment are summarized in Table 4.

The analysis of variance of the growth data shows the greatest necessary difference for significance to be 51.0 grams final body weight. Thus the replacement of horsebean meal of the basal ration with any of the above protein supplements gave a significant improvement in growth. Since 10 chicks in the lot receiving fish meal died through accident, data on the fish meal group were not included in the analysis of variance. However, the survivors showed the highest mean final body weight and therefore it is not unreasonable to assume that fish meal possesses excellent replacement value. From the growth data presented in Table 4 it would appear that fish meal, sunflower seed oil meal, and casein were the most effective complementary sources of protein in the basal ration for purposes of growth, and meat meal, soybean meal and water-treated linseed oil meal follow in decreasing order of value.

The amount of feed required to produce a unit of gain was greatly reduced by supplementing the horsebean meal with fish meal, casein and sunflower seed oil meal, and reduced to a lesser extent by supplementing with meat meal, soybean oil meal and linseed oil meal.

The data recorded on feathering show that the poor feather development and the absence of black pigment, principally on primary and secondary wing feathers and characteristic of the basal ration, were corrected by fish meal, and appreciably lessened by casein, meat meal, sunflower seed oil meal and water-treated linseed oil meal. Soybean oil meal failed to improve either feather growth or feather pigmentation. It is concluded that animal proteins generally, and fish meal particularly, complement the proteins of horsebean seed more adequately than do the proteins of vegetable origin. The exception is sunflower seed oil meal which in this experiment proved almost as effective as fish meal and casein, and was slightly superior to meat meal.

Experiment 2

It is recognized that in practical diets for growing chicks at least 20 per cent of the total protein should be derived from protein of animal origin (23). However, the specific amounts of animal protein required to supplement vegetable protein in poultry rations vary greatly depending upon the particular plant protein supplement used (14). Likewise, condensed fish solubles are reported to be a very rich source of factor or factors usually associated with animal protein and essential in chick nutrition (6, 7, 18).

The second experiment was undertaken to study primarily the relative complementary value of fish meal and meat meal fed alone or in combination at levels yielding 10 and 20 per cent of the total crude protein of the ration.

Condensed fish solubles at levels of 2 per cent and 4 per cent were also added to the basal ration. Because the horsebean meal had been lowered to approximately 25 per cent in the basal ration, soybean meal was used to make adjustments for the different amounts of animal protein supplements required to fix the desired protein ratios. Lots of twenty chicks were fed the experimental diets shown in Table 5 for a period of five weeks.

The results of this experiment are summarized in Table 6. An analysis of variance was carried out and the necessary difference for significance in mean final weights was found to be 33.6 grams. Based on this level of significance, the inclusion of fish meal protein at levels as low as 10 per cent of the total protein in the ration induced better growth than the horsebean meal fed alone or supplemented with meat meal at either of the two levels used in this experiment. Therefore, the improvement of the basal ration observed in lots 4 and 5 can be primarily attributed to the beneficial effects of fish meal. Further improvement was also observed when fish meal was incorporated into the basal ration at level of 20 per cent of the total protein. Condensed fish solubles when fed at levels of 2 per cent (about 3.5 per cent of the total protein) or 4 per cent of the total ration, gave results equal to

TABLE 6.—RELATIVE COMPLEMENTARY VALUE OF FISH MEAL, MEAT MEAL, AND CONDENSED FISH SOLUBLES TO THE HORSEBEAN SEED MEAL PROTEIN

Lot No.	Animal Protein Supplement	Per cent of the Total Protein	Final Weight at 6 Weeks[1]	Feed: Gain Ratio
			Grams	
1	Basal Ration	—	299.3	3.08
2	Meat Meal	10	297.5	3.01
3	Meat Meal	20	325.5	2.92
4	Meat Meal plus Fish Meal	5 5	324.7	2.89
5	Meat Meal plus Fish Meal	10 10	346.8	2.94
6	Fish Meal	10	338.8	2.82
7	Fish Meal	20	355.6	2.83
8	Condensed Fish Solubles	2% of the Ration	346.6	3.05
9	Condensed Fish Solubles	4% of the Ration	352.2	2.93

[1] Necessary difference for significance in final weight is 33.6 at the 0.05 level.

TABLE 7.—EFFECT OF SUPPLEMENTARY SYNTHETIC AMINO ACIDS[1], CHOLINE AND FOLIC ACID[2] IN THE BASAL HORSEBEAN SEED MEAL CHICK RATION

Lot No.	Supplement Added	Per cent of Dietary Requirement	Final Weight at 5 Weeks[3]	Feed: Gain Ratio	Feathering[3]	
					Growth Score	Achroma Score
			Grams			
1	Horsebean Seed Meal	—	142.5	3.69	3.1	2.6
2	Fish Meal	10% of Total Protein	238.8	2.32	2.0	1.6
3	Glycine (Gelatin)	50	157.4	3.45	2.7	2.8
4	dl-Tryptophane	25	149.0	3.52	2.7	2.6
5	dl-Methionine	50	246.1	2.69	0.6	1.2
6	Choline	100	187.2	3.04	3.7	2.6
7	dl-Methionine plus Choline	25 100	264.7	2.53	0.5	0.5
8	1.(+) Lysine	25	172.1	3.18	2.8	3.1
9	Folic Acid	100	146.6	3.39	2.9	2.3

[1] Supplied by Merck & Co. Ltd., Montreal, Canada.
[2] Supplied by Lederle Lab., Pearl River, N.Y., U.S.A.
[3] Necessary differences for significance in final weight and feathering are 33.4 and 0.7 respectively at the 0.05 level.

fish meal when the latter supplement contributed 2 per cent of the total protein. The inclusion in the basal horsebean ration of meat meal protein to a level of 20 per cent of the total protein failed to show a statistically significant beneficial effect. It is concluded from these observations that fish meal and condensed fish solubles were more suitable than meat meal as supplements to a practical horsebean seed meal ration for chicks.

Some difficulties were encountered in the scoring of feather growth and feather pigmentation. However, the high correlation coefficients obtained from data of the next experiment between body weight and feather growth score (−.872), body weight and achroma score (−.748) and feather growth and achroma scores (.898) indicate that rations which induced better growth of chicks also produced better feathering.

Experiment 3

Some legume seed proteins, e.g., soybean and pea proteins, are deficient in available sulphur-containing amino acids which are essential for the growth of chicks (3, 9). Consequently, significant improvements in the growth of chicks were observed when dl-methionine was added to practical chick starting rations containing large amounts of soybean and pea protein (8, 9, 11). On the other hand, definite biological relationships between choline, homocystine, cystine and methionine were demonstrated in the chick (1, 2, 13, 16). The addition of choline alone in certain instances enhanced the nutritive value of a soybean oil meal ration (11).

In the case of basal horsebean seed ration, it cannot be precluded that the lack of essential amino acids other than the sulphur containing groups may contribute to the poor biological response demonstrated thus far. Therefore, a variety of purified nutrients, viz., choline, folic acid, 1 (+) lysine, dl-methionine, glycine (gelatin) and dl-tryptophane were added to the basal horsebean ration in order to determine more specifically the major nutritive deficiencies of the horsebean meal. Fish meal was also fed as a comparative supplement.

The supplements were added to the basal horsebean ration at levels given in Table 7. Ten chicks per lot were fed the experimental rations for four weeks and the results of this experiment are summarized in Table 7.

An analysis of variance showed the necessary differences for significance to be 33.4 grams for body weight and 0.7 for feathering. Based on these levels of significance, supplementary fish meal, dl-methionine, choline, dl-methionine plus choline in the basal ration induced better growth than the raw horsebean meal alone. The abnormal feathering caused by the raw horsebean meal was corrected almost completely by supplementing the basal ration with dl-methionine alone, or dl-methionine plus choline. Some improvement of the feathering was also observed when fish meal was included in the horsebean ration, but this did not equal that produced by the synthetic supplements above. The amount of feed required to produce a unit of gain was greatly reduced when the horsebean meal was supplemented with fish meal, dl-methionine alone, or dl-methionine plus choline. No improvement of the horsebean seed meal was observed upon supplementation with glycine, dl-tryptophane, lysine, or folic acid. It is concluded that the primary deficiency of the horsebean meal protein lies in the low content of sulphur-containing amino acids.

SUMMARY

In a series of experiments with male Barred Plymouth Rock chicks, it has been demonstrated that horsebean seed meal with proper supplementation can serve as a basic source of vegetable protein in a chick starter ration. Without proper supplementation, horsebean meal is deficient in nutritional factors essential for optimum body growth and proper feathering in the chick.

When one-half of the crude protein supplied by 35 per cent horsebean meal to the basal ration was replaced by the following protein supplements, fish meal, sunflower seed meal, casein, meat meal, soybean meal and linseed oil meal, growth was significantly increased. In most instances, this was accompanied by better feather development. Fish meal and sunflower seed meal demonstrated superior supplementary value; casein supplemented the basal ration more adequately than soybean meal and linseed oil meal and meat meal proved better than linseed oil meal. A modified horsebean meal basal ration containing soybean meal was significantly improved by the addition of fish meal, but meat meal was without effect. On the basis of weight, condensed fish solubles and fish meal proved to have about equal value in correcting deficiencies of the basal meal ration.

By supplementing the basal horsebean ration with synthetic vitamins and amino acids, it was demonstrated that the primary deficiency of the horsebean seed protein lies in the low content of sulphur-containing amino acids. Supplementary dl-methionine alone was highly beneficial. When choline + dl-methionine were added to the basal horsebean ration, the deficiency was corrected to a very large measure as shown by improved body growth and restoration of normal feathering in the chicks.

ACKNOWLEDGMENT

The financial assistance of the Quebec Scientific Research Bureau which has made this study possible is gratefully acknowledged.

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BORON IN RELATION TO THE CULTURE OF THE PEACH TREE[1]

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[Received for publication May 8, 1950]

Although the literature covering the role of boron in the growth of agricultural plants is extremely extensive, little has been found describing the use of this element in connection with peach trees when grown under field conditions. It is the purpose of this paper to describe boron deficiency and excess symptoms on peach and to relate these to the results of chemical analyses of tree tissues.

McLarty (5) reported a die-back on peach as a symptom of boron deficiency, but gave no detailed description. Davidson (1) stated that boron deficiency rarely occurs in peach orchards but that it has been found in commercial orchards in the northwestern United States. The affected trees were characterized by their leaves, which were small, thick, misshapen, and brittle.

Symptoms of boron deficiency on peach when grown in sand culture have been described by Weinberger and Cullinan (8). They found that peach trees not supplied with boron were rapidly and severely affected with disorders. Soon after a minus boron treatment was started, in one of their experiments, a dark green water-soaked spot appeared on every growing tip about one inch back from the end and exuded gum. Gradually this spot enlarged, became brown and necrotic, and girdled the growing point. The leaves beyond the affected area wilted and died and later the tip slowly died back for some distance. Numerous lateral buds below the dead tissue started growth, but these also were similarly affected after developing a few small narrow leaves. Older leaves developed small, irregularly shaped, necrotic areas, which later dropped out leaving a perforated leaf. Most of the leaves were somewhat chlorotic, and defoliation, which occurred early, proceeded from the tip toward the base and included most injured leaves. The bark became brown and rough, with many small cork-like growths. The root system was very poorly developed, and consisted chiefly of fibrous roots.

The symptoms caused by an excess of boron on peach have been described by various investigators. Haas (3), when using a culture solution containing 2 p.p.m. boron, found that the leaves burned severely along the entire margins, became wrinkled, and later splitting occurred as the drying out of the margins increased. In both experimental cultures and in the field, Eaton (2) observed boron injury as a tendency for the bark of twigs above some of the leaf axils to turn brown and split open, either with or without the extrusion of gum. Often the bark injury encircled the twig and caused death of the tissues above. When injury was sufficiently severe, the leaf tissues died irregularly along a narrow margin with an accompanying or preceding cupping of the leaf, and the breaking of the restricted margins gave the affected parts a ragged appearance. Weber (7) studied

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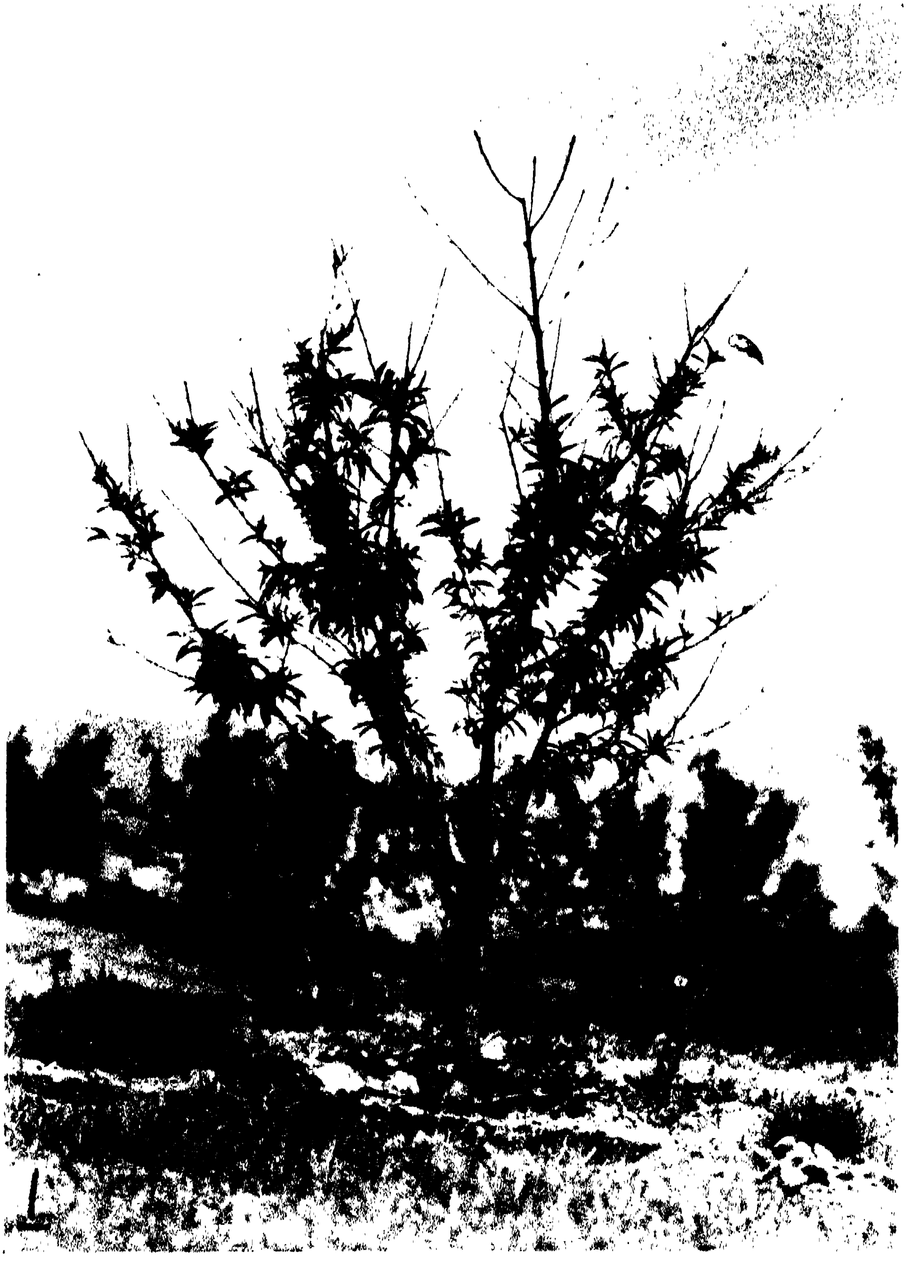


FIGURE 1. Peach tree showing moderate die-back in the spring on twigs of the previous year's growth due to boron deficiency.

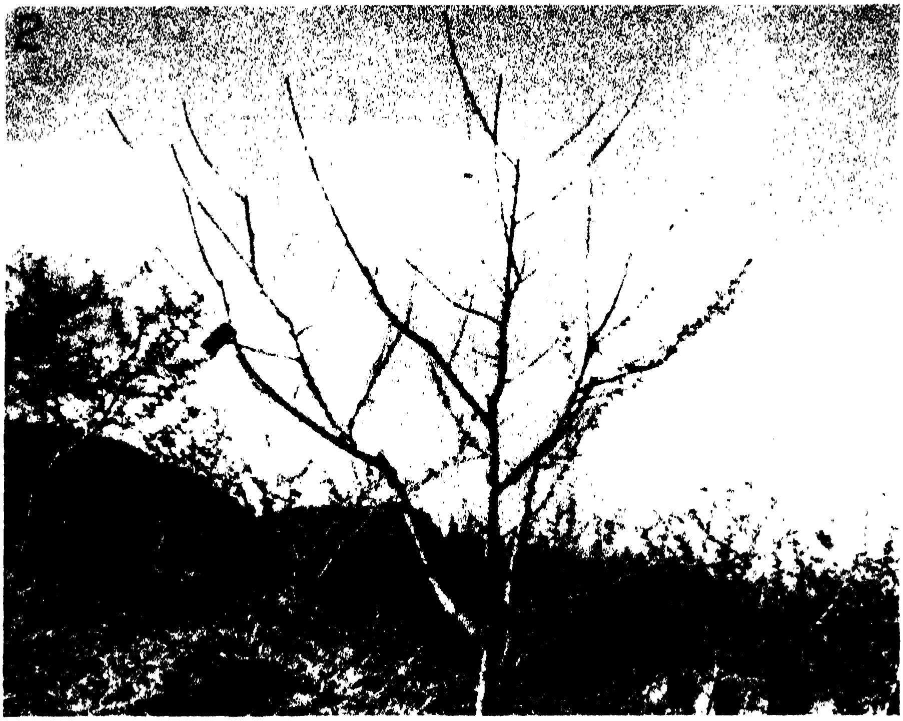


FIGURE 2. Peach tree showing extreme condition of die-back. Most of the limbs and branches have died.

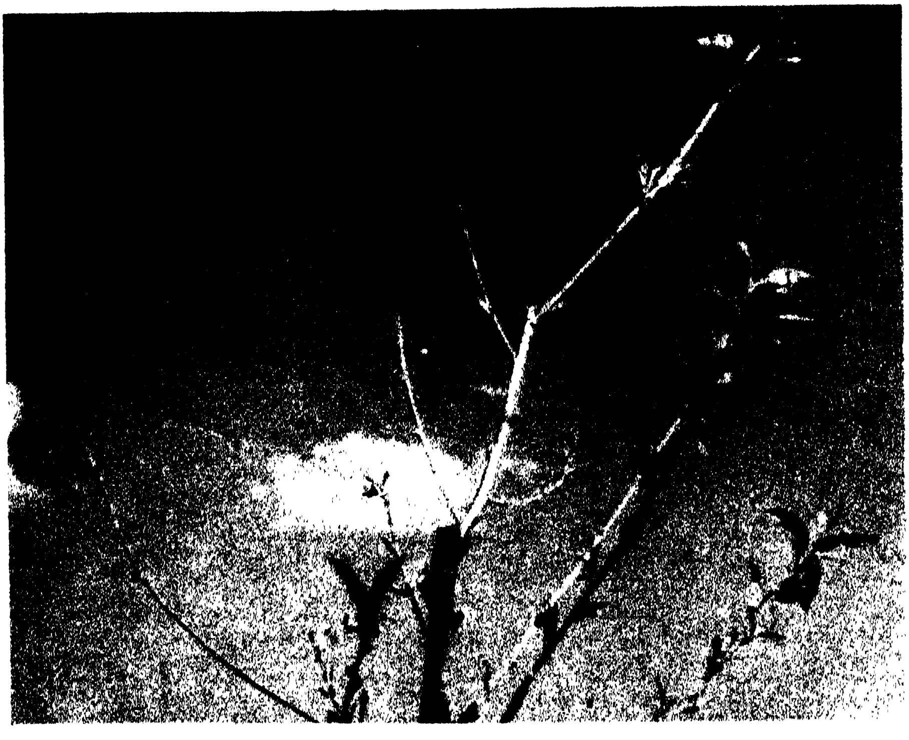


FIGURE 3. Tufted appearance of twigs on a peach tree as a result of a deficiency of boron.

the histological characteristics of the peach when grown in toxic concentrations of boron. He described in detail abnormalities found in peach stems when trees were grown in a culture solution containing 6 p.p.m. of boron and reached the conclusion that such abnormalities were in general similar to those which could be attributed to other causes.

SYMPTOMS OF BORON DEFICIENCY

As the symptoms of boron deficiency as encountered under field conditions in the Okanagan Valley in British Columbia do not correspond to those reported by investigators using sand cultures, the details are presented for comparison. The outstanding characteristic is the dying-back in the spring of twigs and branches on trees which had grown normally the previous season. There is no preliminary warning of the impending attack, for, during the preceding dormant period, the development of both leaf and flower buds corresponds with those on unaffected portions of the tree. No necrotic or atrophied tissues can be found in bud, spur or stem, and no gumming is in evidence. The earliest symptom is failure of both leaf and flower buds to "break" normally when growth starts in the spring. They remain alive until full blossom time, when the majority of them slowly turn brown and die. The stem tissues may remain normal in appearance for as long as from two to three weeks after the buds have died.

Wide variations occur in the severity of attack. The condition most frequently encountered is a dying-back of the previous season's terminal growth over the whole or on one portion of a tree (Figure 1). An extreme condition, but one which has frequently been observed, is illustrated in Figure 2—no or almost no buds "break" on an entire tree. The least recognizable symptom is the failure of the lateral buds to develop on a single twig of the previous season's terminal growth. Most susceptible to attack are the lateral buds on shoots that made rapid growth the previous season, and here the flower buds may succumb while the leaf buds survive. When the lateral buds on a twig are killed, and not the terminal bud, the subsequent growth gives to that twig a tufted appearance (Figure 3). Affected flower buds rarely develop their flowers, but affected leaf buds may survive and send out a retarded shoot growth. Occasionally this shoot may wither and die, but usually it lives and produces a healthy growth during the season. However, the first leaves that develop may be small, narrow, and with edges rolled inward. Wherever affected leaf buds survive, the stem tissues also remain alive and that portion of the tree recovers. When both the flower and leaf buds are killed the adjacent stem tissue usually dies, but sometimes adventitious buds develop and the twig survives.

Boron deficiency die-back has some symptoms in common with those caused by winter injury and it is frequently difficult to differentiate between them. The outstanding difference which has been observed is that with winter killing there is always a death of tissue, either in the stem, spur or blossom, which takes place shortly after their exposure to the killing temperature, whereas, with boron deficiency, there is no evident injury until after growth has started in the spring.

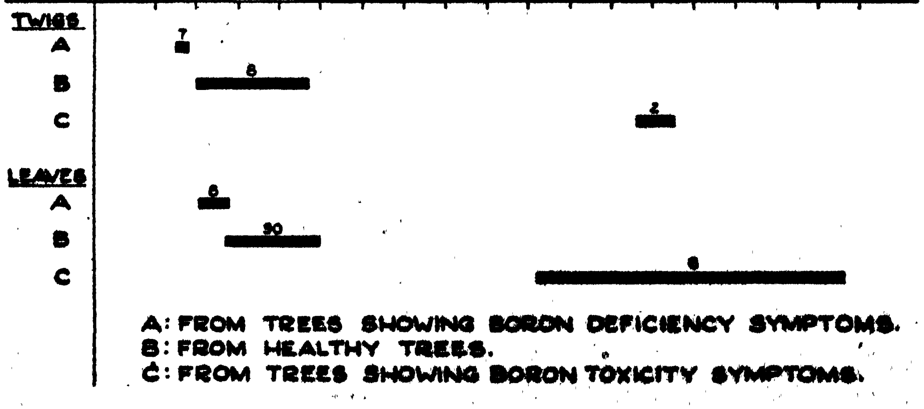
SYMPTOMS OF BORON EXCESS

Boron excess symptoms which have been observed in the field have usually appeared in the summer following a fall or spring in which an excessive application of boric acid was made. In severe cases, the tips of the current season's branches wither and die-back (Figure 4), cankers develop along the stems, and much gumming is in evidence (Figure 6). With less severe injury, small necrotic areas develop in the leaves along the midrib and small cankers develop on the underside of the midrib and in the petiole (Figure 5). The necrotic areas drop out after from 10 to 15 days, leaving perforations along the midrib. The symptoms are frequently associated with a brittleness of the leaves and a partial defoliation. Fruits on trees showing boron toxicity symptoms are malformed and kernal development is poor (Figure 7). Only in a few orchards have trees been killed outright and most trees exhibit these symptoms for just the one year immediately after an excessive application of boric acid. Under irrigation, the trees usually return to normal growth in the following season. Leaf symptoms, similar to those described, have been induced on 2-year-old peach trees in the first season that they were grown in sand culture out-of-doors when the nutrient solution contained 5 p.p.m. boron.

CHEMICAL ANALYSIS

Samples of twigs and leaves were collected over a period of two years from reputedly healthy trees and also from trees showing either boron deficiency or boron toxicity symptoms. The samples were dried, ground and analysed for total boron by the turmeric colorimetric method which has been described in detail by Naftel (6) and Haas (4). The results of these analyses are summarized in Figure 8, where the heavy horizontal lines represent the range of boron concentration found in tissues of a given category and the numeral above the line is the number of samples analysed in this category.

FIG. 8.—THE RANGE OF BORON CONTENT OF PEACH TWIGS AND LEAVES. (NUMERALS ABOVE HEAVY LINES INDICATE THE NUMBER OF SAMPLES ANALYSED IN EACH GROUP OF TWIGS OR LEAVES)



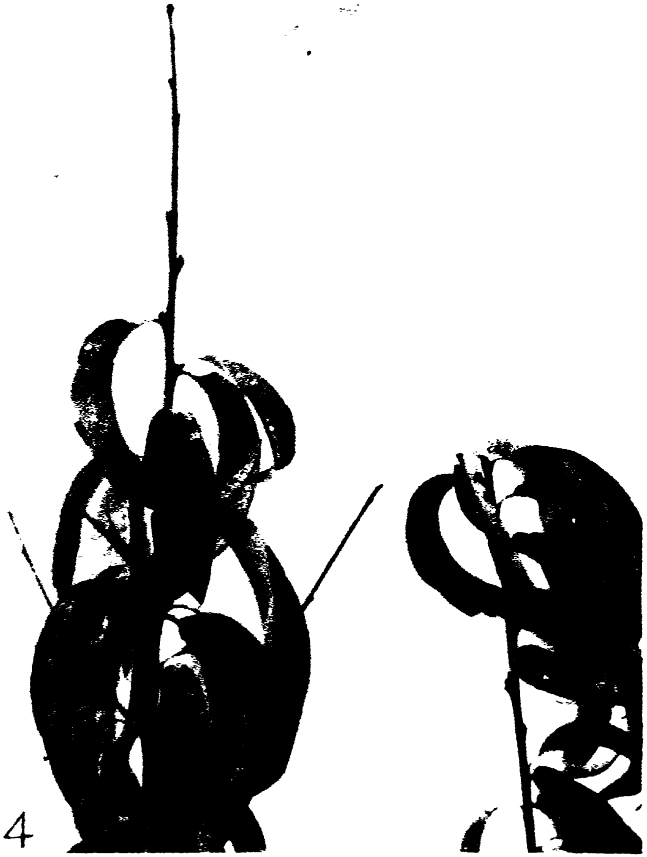


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FIGURE 4. Twigs of a peach tree showing die-back in the summer on the current year's growth due to boron toxicity. (Compare with Figure 1).

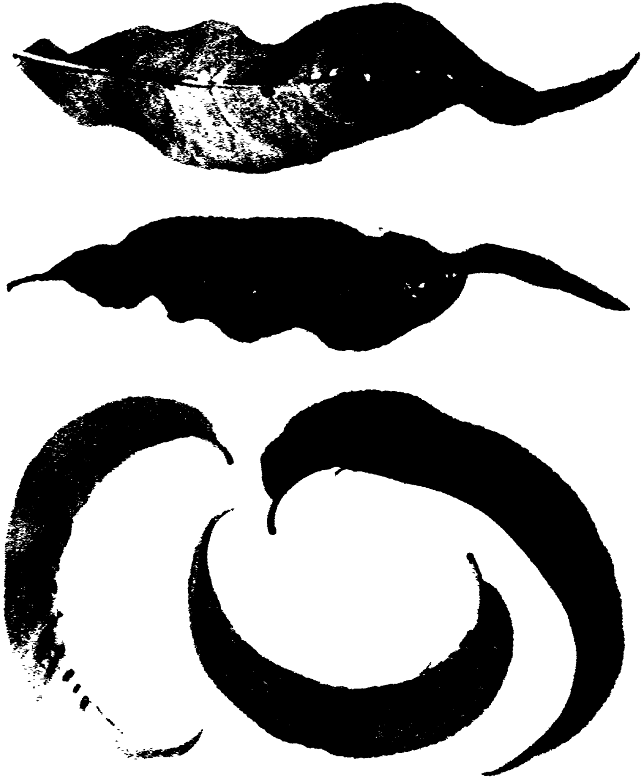

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FIGURE 5. Leaves of a peach tree showing injury due to boron toxicity. Note small necrotic areas along the midrib and cankers on the underside of the midrib. Some of the necrotic spots have dropped out.

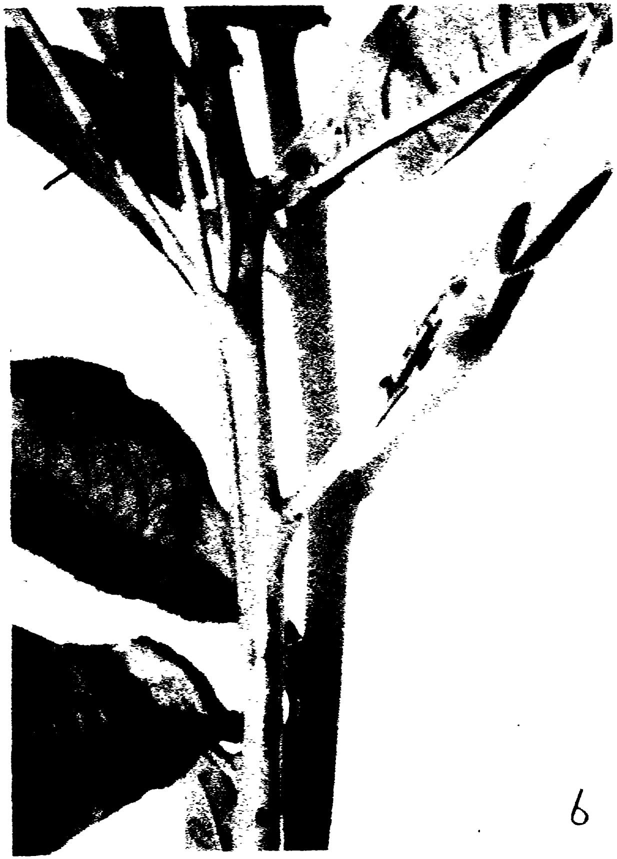

Figure 6. Example of severe boron toxicity on peach twigs; cankers are present along the stem and gumming *(arrow)* is in evidence.

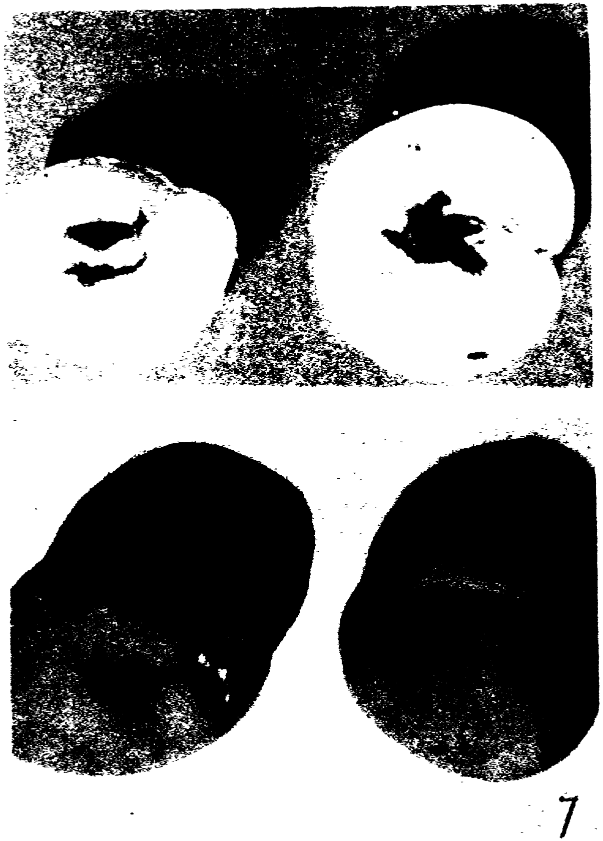

Figure 7 Boron toxicity in fruit. The fruits are malformed and kernel development is poor.

SUMMARY

1. The outstanding characteristic of boron deficiency on peach is the dying-back in the spring of twigs and branches on trees which grew normally the previous season.

2. The symptoms of boron toxicity that are most readily recognized are small necrotic areas along the midrib of the leaf and small cankers on the underside of the midrib.

3. The results of the chemical analyses indicate that, if the boron content on a dry weight basis of the leaves is less than 20 p.p.m., or that of the twigs is less than 10 p.p.m., boron deficiency symptoms are very likely to be present. If the boron content of leaves is greater than 90 p.p.m., and that of twigs is greater than 110 p.p.m., boron toxicity symptoms will most probably be apparent. Normal leaves and twigs contain 17 to 40 p.p.m. and 10 to 37 p.p.m., respectively.

ACKNOWLEDGMENTS

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A MECHANICAL SHAKING DEVICE[1]

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The recent advent into North American commerce of several kinds of seed-treating machines, for applying fungicides in dry or wet form to the seed, has given rise to a need for a laboratory seed-treater which gives standard "mixes" on the basis of which the efficiency of commercial machines could be determined. Existing laboratory seed-treating devices have limited use, being designed for either dusts or for fluids. In some cases, when both can be applied by the same machine, there are various drawbacks limiting the machine's use. The device described here (Figure 1) has the advantage of being able to prepare as many as six one-pound mixes at a time, so that a considerable quantity of seed can be handled. Dry or wet disinfectants may be applied simultaneously and an exact count may be obtained of the number of shakes given the mixtures.

The present machine was designed and built in the instrument shop of the Dominion Laboratory of Cereal Breeding, Winnipeg, for the purpose of mixing seed and fungicide. From observations made over a period of several years by J. E. Machacek, of the Dominion Laboratory of Plant Pathology, Winnipeg, it appeared that the best mixtures were obtained by putting about one pound of seed in a half-gallon Mason jar and then holding the jar at about 45°, moving it rapidly with a rotary motion of the hand through approximately a six-inch circle. This gave the seed a sliding, tumbling motion, and left no unmixed "pockets" in the jar. This motion imparted by hand shaking is duplicated in the machine described below but without the variations resulting from manual operation.

This machine consists of three essential parts: a rack which holds the Mason jars at an angle of 45° in semi-cylindrical troughs lined with sponge rubber; a motor-driven mechanism which moves the rack 300 revolutions per minute through a three-inch circle, and a resettable counter attached to one of the crankshafts which drive the rack. All these components are mounted on a rigid, angle-iron frame.

The moving rack is built of 22-gauge sheet iron welded to a ⅝-inch steel tubing framework. The troughs for the Mason jars are formed in the sheet iron rack and the sponge rubber cushion is fixed in place with rubber-base adhesive. The jars are held firmly in place by means of straps of rubberized webbing equipped with adjustable buckles. Where a uniform size of jar is always used, a sponge-lined steel strap with luggage clasps attached will allow faster manipulation. The whole rack is attached to two crankshafts, to one by two rigid tubular legs with brass bearings, and to the other by two hinged legs with brass bearings. This arrangement, of rigid and hinged legs, reduces the stresses arising from wear or irregularity in assembly. Both crankshafts run in bronze bearings and are driven by bicycle chains and sprockets. With this dual driving arrangement the

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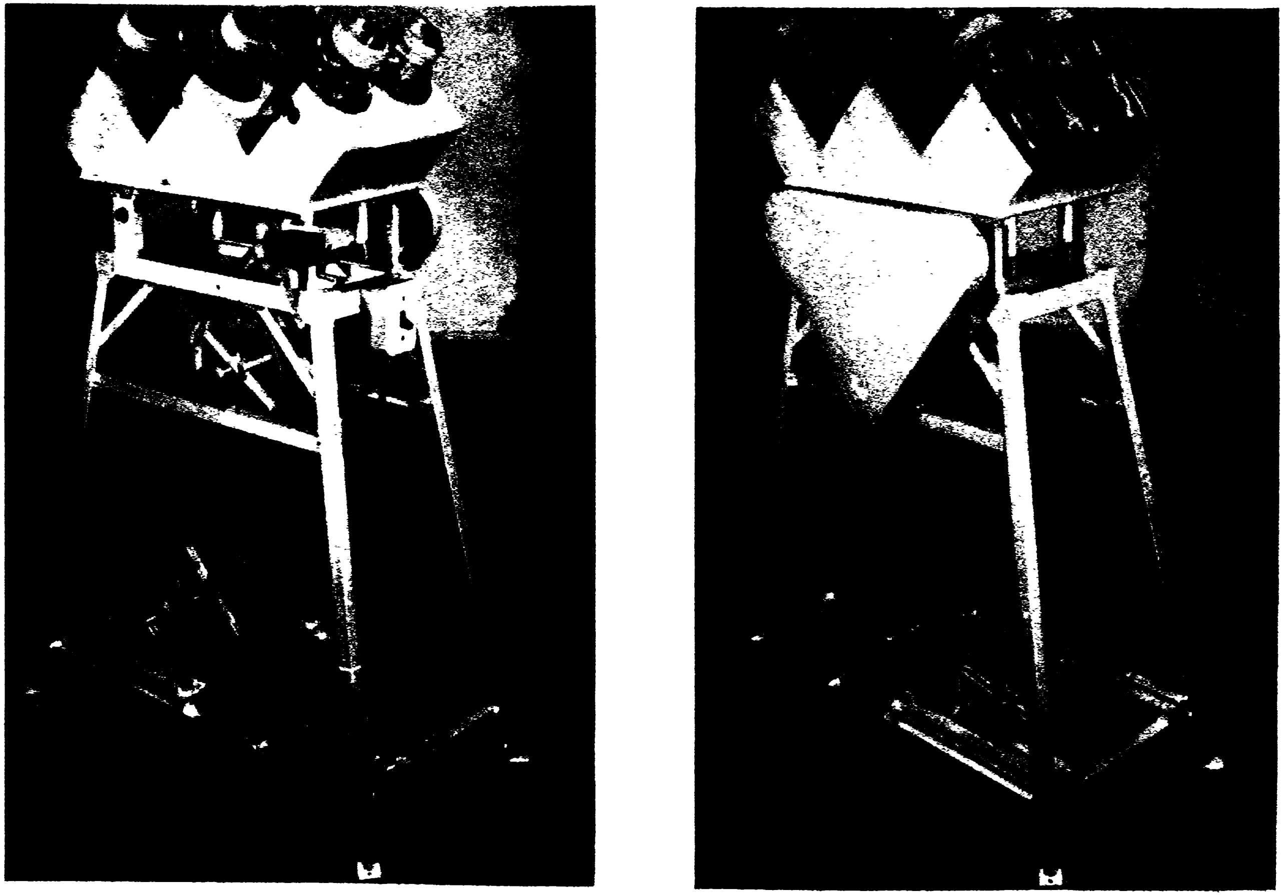

(a) (b)

FIGURE 1. Views of mechanical shaking device.

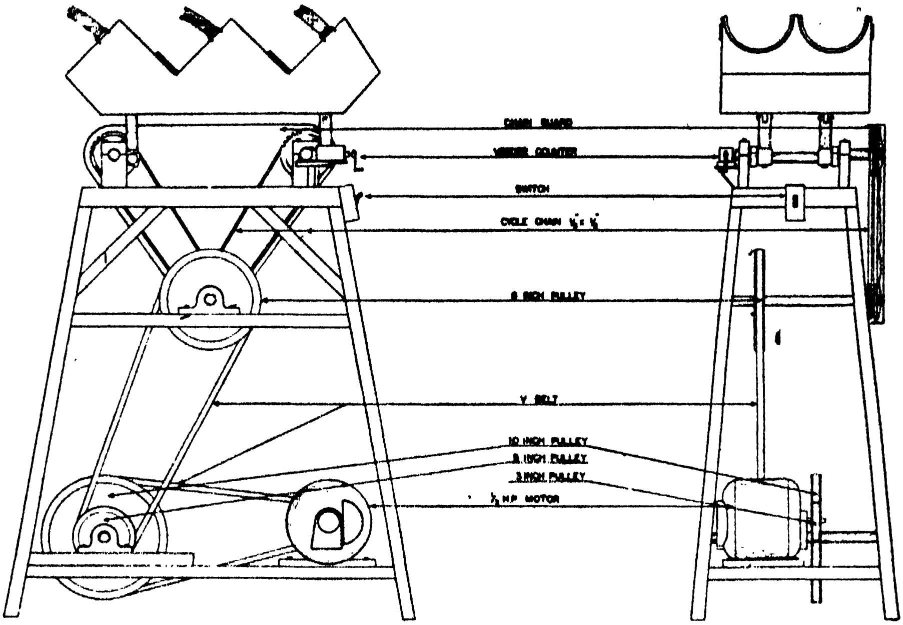

FIGURE 2. Driving arrangement for mechanical shaking device (Scale 13/16 in = 1 foot).

rack will move as required; but if only one drive chain is used, the undriven crankshaft will merely rock to and fro, leaving that end of the rack comparatively unshaken.

The drive for both chains is taken from the second of two countershafts used to reduce the motor speed of 1725 r.p.m. to the 300 r.p.m. required for satisfactory operation. Power is transmitted from the one-third horsepower motor through the two countershafts by V-belts and pulleys. The whole drive arrangement is shown in Figure 2.

A counter with a manual reset which is used to determine the number of shakes each lot of samples receives is located on the main framework below the jar rack. A switch for starting and stopping the motor is also attached to the framework near the counter.

The main framework is fabricated from 1 in. × 1 in. × ⅛ in. angle iron, except the top members which are 1½ in. × 1½ in. × 3/16 in. All joints are welded together.

Our observations show that this machine may satisfactorily be used for other purposes such as mixing inoculum with seed, preparing emulsions, mixing paints, and the like. Where a large number of small samples are to be shaken, interchangeable racks may be used.

While in operation the machine should be bolted to the floor; or if used in a temporary location, the legs should be bolted to planks weighed down with heavy objects such as boxes of sand. Without this anchoring, the machine tends to vibrate to a considerable degree.

A RAPID METHOD OF ESTIMATING SOIL IN RUN-OFF SUSPENSIONS[1]

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[Received for publication May 22, 1950]

INTRODUCTION

In the measurement of soil and water losses resulting from erosion on experimental plots of the Division of Field Husbandry, Central Experimental Farm, Ottawa, Ontario, the run-off is collected in catchment tanks located at the lower end of the plots. Some preliminary results from this experiment have been reported (1). Samples of the soil-water suspensions from the tanks are brought to the laboratory for determination of the amounts of soil and water. The procedure has been to evaporate weighed aliquots of the suspensions to dryness and to calculate the per cent soil present from the weight of the dried residue. Although this procedure, which will be referred to as the "evaporation method", has proved satisfactory in most respects it requires laboratory space and considerable equipment.

Since in some instances erosion experiments may be located in areas where laboratory facilities are not conveniently available, it was decided to investigate the possibility of estimating the amount of soil in the suspensions from the difference in weight between a given volume of water and an equal volume of the suspension. The amount of soil in a suspension may be calculated from the equation

$$P = \frac{100S(X-Y)}{X(S-1)} \qquad \text{Equation 1}$$

where P is the per cent soil in the suspension, S is the specific gravity of the soil, and X and Y are the weights of equal volumes of the suspension and water respectively at the same temperature. It is evident that such a method should be feasible providing the specific gravity of the soil in the various suspensions is relatively constant.

EXPERIMENTAL

(a) Estimation of Specific Gravity of Soil in Suspension

The soil of the experimental area has been mapped as Rideau clay (2). It is described as a very heavy, moderately drained, stone-free soil, slightly to moderately acid in reaction, and is relatively low in organic matter.

Composite samples of the dried residues from the run-off suspensions collected in 1946, for each of four plots, provided a basis for establishing an estimate of the average specific gravity of the soil in the suspensions. The specific gravity of each of these four samples was determined by the method described by Lyon and Buckman (3) except that the air was removed from the soil-water mixtures in the pycnometers by placing them in a vacuum. The results, presented in Table 1, show that the mean specific gravity of the soil material was 2.657 and that the variation from the mean for the individual samples was very small.

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[1] Joint contribution from the Division of Chemistry, Science Service and the Division of Field Husbandry, Soils and Agricultural Engineering, Experimental Farm Service. Scientific contribution No. 182, Division of Chemistry, Science Service.

[2] Assistant chemist, Division of Chemistry, Science Service.

[3] Agronomist, Division of Field Husbandry, Experimental Farm Service.

TABLE 1.—SPECIFIC GRAVITY OF SOIL MATERIAL IN THE RUN-OFF FROM FOUR PLOTS IN 1946

Plot	Specific gravity
5	2.656
11	2.664
20	2.653
21	2.655
Mean.........	2.657

By using the value 2.66 for the specific gravity of the eroded material as an estimate of S in Equation 1, the per cent soil in the suspensions may be calculated without recourse to drying the samples. On this basis, a rapid method of estimating eroded material in run-off suspensions is proposed and will be referred to as the "specific gravity method".

(b) Description and Evaluation of the "Specific Gravity Method"

The procedure for the "specific gravity method" was as follows: A bottle of about 800 ml. capacity, with a ground glass stopper through which a hollow glass tube passed, was constructed out of an ordinary Erlenmeyer flask and a ground glass joint. The glass tube through the stopper was 4 mm. in diameter and extended from the bottom of the stopper to about 2 cm. above the top. The weight and volume capacity of the bottle were determined accurately. The weight of suspension required to fill the bottle was determined and the temperature recorded. The weight of an equal volume of water was calculated from the known capacity of the bottle and the density of water at the temperature recorded. The per cent soil in the suspension was then calculated from Equation 1.

Following the above procedure, the per cent soil in each of 41 suspensions collected from the experimental plots over a period of two years was determined in duplicate. The means of these duplicate determinations, along with the corresponding values obtained with the "evaporation method", are presented in Table 2. The standard error for each method, and the standard error based on the differences between means of duplicate determinations by each method, are included. The two methods gave similar results. The standard errors of ± 0.026 for the "specific gravity method" and ± 0.043 for the "evaporation method" were small. The standard error based on the differences between means of duplicate determinations by each method was ± 0.077. Although this latter error was of a greater magnitude than can be accounted for by the errors within methods, it was not appreciable.

TABLE 2.—PER CENT SOIL IN RUN-OFF SUSPENSIONS BY THE EVAPORATION AND SPECIFIC GRAVITY METHODS

(Means of duplicate determinations)

Plot No.	Date of rain	Soil in the suspensions		Plot No.	Date of rain	Soil in the suspensions	
		Evaporation method	Specific gravity method			Evaporation method	Specific gravity method
		%	%			%	%
1	27–8–49	1.80	1.80	11	24–7–48	4.34	4.49
2	7–7–48	2.62	2.59	11	21–6–49	1.72	1.65
2	25–6–49	0.84	0.89	11	25–6–49	6.62	6.63
2	30–7–49	0.61	0.62	11	27–8–49	4.69	4.75
5	24–7–48	2.49	2.40	11	30–8–49	2.52	2.56
5	25–7–49	2.40	2.36	12	30–7–49	2.45	2.39
5	29–7–49	1.78	1.77	13	7–7–48	6.15	6.30
5	26–8–49	4.28	4.42	13	25–6–49	2.32	2.12
5	13–9–49	2.44	2.47	13	29–7–49	0.91	0.88
6	7–7–48	5.47	5.57	14	30–8–49	4.26	4.31
6	24–7–48	1.53	1.43	18	30–8–49	0.68	0.69
6	21–6–49	1.86	1.71	19	30–7–49	0.69	0.71
6	25–6–49	3.37	3.36	19	30–8–49	1.03	0.91
6	29–7–49	2.24	2.23	20	7–7–48	2.45	2.54
6	29–8–49	4.43	4.49	20	24–7–48	1.05	0.96
6	13–9–49	2.37	2.49	21	7–7–48	2.64	2.60
10	7–7–48	5.55	5.56	21	21–6–49	1.24	1.16
10	25–6–49	3.32	3.30	21	25–6–49	2.25	2.29
10	30–7–49	3.00	3.02	21	14–9–49	1.95	1.97
10	26–8–49	5.30	5.35				
10	14–9–49	3.95	3.97		Mean	2.87	2.87
11	7–7–48	6.06	6.04				

Standard error of a single determination with Evaporation Method ± 0.043.
Standard error of a single determination with Specific Gravity Method ± 0.026.
Standard error based on the differences between means of duplicate determinations by each method ± 0.077.

DISCUSSION

The "specific gravity method" has given essentially the same results as the "evaporation method" under the conditions of the erosion experiment at Ottawa. The specific gravity of the soil in suspensions from experiments located on other soils might vary somewhat from that found in this investigation but it may be readily determined. On soils relatively high in organic matter the specific gravity of the soil in the different suspensions might vary to a greater extent than was encountered in this investigation.

In many erosion experiments a reasonable estimate of the amount of soil lost by erosion is all that is required. In such cases the specific gravity of the soil could be assumed to be 2.65, the average for mineral soils (3), and the "specific gravity method" used to estimate the per cent soil in the suspensions. In this connection it should be noted that a difference of 0.1 between the actual specific gravity of the soil in the suspension and that assumed for calculating would cause an error of only about 2.5 per cent in the estimation of the amount of soil present.

SUMMARY

A rapid method for the determination of the amount of soil in the soil-water run-off suspensions from plots in an erosion experiment has been proposed. The method is based on the difference in weight between equal

volumes of water and the suspension at the same temperature. It does not require laboratory space, and the equipment needed is an ordinary beam balance accurate to 0.1 grams, a specially constructed bottle, and a thermometer.

The method has been compared with the "evaporation method" and has been found to give satisfactory results.

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PACKAGE MEASLES OF TULIPS[1]

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Large losses of tulip bloom were sustained by two greenhouse operators on Vancouver Island through the extensive development of spots on the foliage and petals after the bloom was wrapped in oil paper for shipment to market, a condition known locally as "package measles". The spots appear as small water-soaked areas, less than 2 mm. in diameter, within twenty-four hours after the bloom is wrapped. These spots later become slightly sunken.

A close examination of the growing plants at the time the bloom was being cut failed to reveal any spots or other abnormalities in the foliage, except a general but slight etiolated condition, probably due to a long period of dull weather preceding the appearance of the bloom. The investigation revealed also that it was the practice of the operators to place the exposed stem bases of the packages of wrapped tulips in water for as long as twenty-four hours before placing them in cartons for shipment. Consequently, water condensation within the wax paper packages was suspected as being the cause of "package measles".

EXPERIMENTAL

An experiment was undertaken to ascertain whether or not moisture condensation within the packages was a contributing factor in the development of "package measles". The exposed stem bases of ten packages, each of one dozen blooms, were placed in water for twenty-four hours immediately after they were cut and wrapped. Ten dozen unwrapped blooms were placed in water for twenty-four hours and then wrapped. Forty-eight hours after both lots of bloom had been cut, the packages were opened. The blooms within all the packages bore the typical symptoms of "package measles", but, without an exception, the spotting was less extensive and less conspicuous on the blooms that had been placed in water twenty-four hours before they were wrapped. Moisture condensation within the packages appeared to favour the development of the disease.

Another experiment was designed to ascertain whether or not the disease was confined to the original two greenhouses whose operators had reported large losses. Ten dozen tulips from each of the two houses were packaged immediately after being cut and the exposed stem bases were placed in water for twenty-four hours. A like number from two houses whose operators had not reported losses were handled in the same manner. Twenty-four hours after wrapping, typical symptoms of the disease were found on the bloom in the packages from the original houses, but none on the bloom from the second pair of houses, although the environment in both pairs of houses seemed to be identical. It was evident, therefore, that moisture was not directly responsible for the trouble.

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The discovery of some plant debris, covered with conidiophores of a *Botrytis* sp. in the original houses, served as a clue to the cause. The operators of these two greenhouses had allowed considerable plant debris, chiefly tulip foliage, to accumulate under the benches, and considerable portions of the debris were covered with a fur of conidiophores of the *Botrytis* sp. Spore measurements indicated that the fungus was a form of *B. cinerea* rather than of *B. Tulipae.* A spore suspension was prepared from the spore-bearing parts of this debris by shaking the parts in water. This spore suspension was sprayed upon the tulips in a house the blooms from which had not shown the disease. Ten dozen blooms were then cut, wrapped, and the stem bases placed in water for twenty-four hours. The typical symptoms of "package measles" developed within that time in all ten packages.

Characteristic spots of experimental and of natural "package measles" on the foliage were examined after clearing in lacto-phenol and staining, but in few cases could fungus mycelia be detected in the lesions that had developed within twenty-four hours at approximately 45° F. However, by maintaining such bloom for three or four days in a humid atmosphere, no difficulty was experienced in detecting mycelia and in isolating *B. cinerea* from the lesions. The spotting of foliage with little evidence of fungus penetration has been described by Wilson (1) in connection with his study of chocolate spot of broad bean, a disease also caused by a form of *Botrytis cinerea.*

An experiment was then undertaken with a view to developing a control measure through the prevention of the growth of the *Botrytis* spores on the foliage, for this growth appeared to be the direct cause of "package measles". The tulip plants in one of the original houses were divided into a number of lots and each was sprayed or dusted with a different fungicide, a dozen or more having been used. The bloom was then cut, wrapped, and placed in water, as in the first experiments. Ten packages from each treatment were taken. The opening of the packages after twenty-four hours revealed that the majority of the sprays and dusts left undesirable residues on the foliage and petals, or in themselves caused injury, particularly to the petals. Two thiram sprays, one of Dutch and the other of American manufacture, at 0.5 per cent concentrations, caused foliage and petal injury and failed to prevent the development of "package measles". Ferbam (Fermate) and zineb (Parzate), at the same concentrations, left conspicuous, and therefore undesirable, residues on the foliage, although both were reasonably effective in preventing the development of "package measles". Folosan dust (20 per cent pentachloronitrobenzene) prevented the development of the characteristic symptoms, but the dust on the foliage was sufficiently conspicuous to mar the appearance of the bloom. Nabam (Dithane), as a 0.5 per cent spray, was the most promising. When the ten packages receiving this treatment were opened twenty-four hours after the sprayed blooms had been cut, wrapped, and placed in water, symptoms of "package measles" were not evident and little spray injury could be detected.

Inquiries made after the above-mentioned experiments were carried out revealed that "package measles" has been of not infrequent occurrence in other greenhouses and in other years. The principal source of infection appears to be tulip debris left under greenhouse benches. On this debris, abundant inoculum of *Botrytis cinerea* is produced during periods of humid dull weather. It is during and for a time following such weather that the disease is prevalent on packaged blooms. The practice of thorough greenhouse sanitation would appear to be an adequate preventive measure.

SUMMARY

Large losses were sustained by two greenhouse operators through "package measles" of tulip bloom, a disease characterized by an extensive spotting of the foliage and petals and which develops only after the bloom is cut, and wrapped in wax paper for market. Moisture within the packages was found to be a contributing factor, but the primary cause was traced to contamination of the bloom prior to cutting with spores of a form of *Botrytis cinerea* that originated on plant debris beneath the greenhouse benches during periods of dull, humid weather. Non-contaminated plants when sprayed with a spore suspension and immediately cut and wrapped developed typical symptoms within twenty-four hours at 45° F. Symptom development on the bloom in the packages was prevented by spraying the plants prior to bloom cutting with a 0.5 per cent solution of nabam.

REFERENCE

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THE LOGICAL PRESENTATION OF IDEAS*

Many of the mistakes in scientific papers involve errors in logic. Their avoidance depends chiefly upon a thorough understanding and careful analysis of the ideas that are presented. The following rules apply to some of the most obvious, and yet commonest, mistakes of this type.

1. *Requisites of a good hypothesis.* Is it new? Is it worthy of consideration? (*a*) Do not present as new a hypothesis that really is old. But defective hypotheses of others may be improved, and presented as modified hypotheses. (*b*) The hypothesis should give promise of explaining facts or relations that have not hitherto been explained. (*c*) The hypothesis should be consistent with itself and with well established facts and principles. (*d*) Do not stress one hypothesis when another, or others, would fit the facts equally well. Employ the method of multiple working hypotheses. (*e*) Do not give a complex hypothesis when a simpler one would fit the facts equally well. (*f*) It is rarely useful to propose a hypothesis that cannot be tested or verified. (*g*) The hypothesis should aid the prediction of new facts or relations.

2. *Illusions.* (*a*) Be careful not to draw conclusions from data involving errors of observation, errors in arithmetic, compensating errors, systematic and personal errors. (*b*) Do not use mathematical formulae without clearly understanding their derivation and all the assumptions involved. (*c*) Be cautious in comparing conclusions based upon experiments in which the influential conditions have been improperly controlled, and therefore, not duplicated. (*d*) Guard against drawing an illogical conclusion. (*e*) Avoid confusing facts with opinions or inferences, not only in the investigation itself but also in preparing results for publication.

3. *Too broad generalization.* (*a*) Do not draw a conclusion from too few data, nor too broad a conclusion from a limited series of data. (*b*) Be careful in drawing conclusions that are based on extrapolated curves. (*c*) Guard against failing to qualify a conclusion, so as to show the limits within which it applies, or the variation which is to be expected. (*d*) When you indulge in speculation, be sure that you, and your reader, know that it is speculation.

4. *False relation between cause and effect.* (*a*) Do not infer merely because one thing has followed another that it is the effect of the other. (*b*) Do not argue that causes are the same because identical or indistinguishable effects have been observed. A certain phenomenon may have one cause in one case and another cause in a second case. (*c*) Be careful in making inferences by analogy. If two cases resemble each other in certain particulars, it is not safe to infer resemblance in another particular that has been observed in only one of them. (*d*) If two processes have the same mathematical expression (or yield the same sort of graph when plotted), it does not necessarily follow that the processes themselves are essentially alike.

5. ***Prejudice.*** (*a*) An attitude of intellectual honesty and devotion to truth is the foundation of scientific work. (*b*) Guard against prejudice; do not be influenced by preconceived opinions. (*c*) Do not decline to admit evidence because it necessitates an unwelcome conclusion. If a

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* Reprinted from "Preparation of Scientific and Technical Papers" by Trelease and Yule. 1937.

conclusion is unwelcome, it is a sign something is wrong with one's mental attitude. (*d*) Biting, caustic comments are almost sure to be regretted later, and they almost invariably weaken the effect of one's arguments.

6. *Ambiguity of terms.* (*a*) Guard against misunderstandings of language. (*b*) Define terms as clearly and precisely as possible. Do not use technical terms, especially in a field not strictly your own, unless you are certain of their precise meaning, or unless their use has been checked by a specialist in the field. (*c*) Do not use a term in one sense in one part of your reasoning and in another sense in another part. (*d*) Do not mistake a general for a specific use of a term. (*e*) Be very critical of statements containing the words "cause", "determine", "control," "influence", "result", "effect". Distinguish carefully between such words as "force", "agency", "process".

7. *Missing the point.* (*a*) Do not ignore the question, evade the issue, or argue beside the point. Define clearly the points at issue. Try to determine the crucial point that will really decide the discussion. (*b*) Guard against reasoning that may correctly prove something but not the thing which you think it proves.

8. *Begging the question.* (*a*) Do not base a conclusion on an unproved proposition. (*b*) Avoid arguing in a circle—drawing a conclusion that merely states the assumptions in other words. (*c*) Do not assume the truth of a proposition that is not proved and may be false. (*d*) Do not assume that a certain thing is true because a prominent authority has said it is true. (*e*) Do not assume that a proposition is untrue because you are able to disprove the arguments that have been used to support it; there may be other, valid arguments that make it true.

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THE OCCURRENCE OF *HELMINTHOSPORIUM SATIVUM* IN RELATION TO PRIMARY INFECTIONS IN COMMON ROOTROT OF WHEAT[1]

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INTRODUCTION

Common rootrot is a name given to a complex manifestation of disease in cereals, resulting from the invasion of the crown and roots by certain fungi. *Helminthosporium sativum* P.K. and B. is considered of first importance in this type of disease in wheat and barley. It is known to be widely distributed in the extensive grain growing areas of Western Canada. In this paper, the authors will endeavour to bring up to date observations on the distribution of the pathogen, particularly in Saskatchewan, and present some recent data on primary infections.

A rather extensive literature review was made in seeking recorded observations on primary sources of disease. Bolley (3) was obviously aware of the prevalence of lesions on the wheat plant at, or near, the soil line; and he illustrated (p. 20) a typical lesion on a young wheat plant. He considered that most of these lesions were caused by *H. sativum*, and suggested that the fungus comes from stubble pieces or infected seed. He stressed the importance of diseased seed.

Seed, as a source of infection, was reported by Louise J. Stakman (21), who also mentioned the probability of infections from the soil. Infected seed and infested soil were stated to be sources of primary infections by Christensen (4), who conducted greenhouse and field experiments with wheat and barley. He noted the abundant sporulation from lesions and considered wind the chief factor in dissemination. In her studies of *H. sativum*, Dosdall (6) made some inoculations in pot experiments by pouring a conidial suspension over the surface of the soil. A similar method of inoculation was employed by McKinney (13). The method is of interest to the authors, as will be seen later. Christensen (5) observed primary lesions on wheat and barley sown at intervals from April to October. In Saskatchewan, Sallans (14) found that lesions appear on the subcrown internode of wheat in greatest abundance, commencing early in July. It was well known to most investigators of common rootrot that seedlings growing in infested soil soon became infected; but the source, whether conidia, or mycelium in the soil, or pieces of stubble in which the fungus was established, was not easily determined.

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Shortly after this period, there followed the pioneering research of Sanford and Broadfoot (17) and Henry (9), which showed the influence of soil microorganisms in suppressing root disease pathogens. Their work placed the interpretation of cereal root pathogenesis on a much sounder basis. Henry (10) later came to the conclusion that conidia of *H. sativum* were very rare in Alberta soils. He thought this fungus must exist there in the form of mycelium. In his earlier work (8) also, he was unable to isolate it from soil. Bisby, James, and Timonin (1, 2), in extensive isolations from Manitoba soils by the plate method, obtained only a few colonies of *H. sativum*. By this method, they were unable to determine whether the colonies originated from conidia or mycelial fragments, or from both. Generally speaking, the importance of conidia, in nature, was not stressed in regard to primary lesions, although Christensen (4) emphasized their importance in dissemination. Diseased seed, however, was considered a very important source, and may explain the common tendency for the development of various methods of seed inoculation.

OCCURRENCE AND DISTRIBUTION IN SASKATCHEWAN

Several years ago, isolation work was conducted in an effort to determine the distribution of root-disease fungi, chiefly *Helminthosporium sativum* and *Fusarium spp.* This work was reported in the Dominion Botanist's Reports for 1927 to 1930, inclusive (18). Wheat specimens were received from Morden, Winnipeg, Indian Head, Saskatoon, Swift Current, and Edmonton. The results showed that these fungi were prevalent in Manitoba, Saskatchewan, and Alberta. There appeared to be a seasonal fluctuation in the occurrence of *H. sativum*. Regional differences were inconsistent for the six source locations, with one exception: the Edmonton material invariably gave fewer *H. sativum* isolates. Isolation work by Simmonds and Ledingham (19) showed that infections of *H. sativum* were pretty well confined to the crown and adjacent tissues. These findings directed our attention to surface litter, such as cereal stubble, as a possible source of infection.

This approach was explored by Ledingham (12). He collected two soil samples from each of seven different rotations and, in addition, two samples from virgin sod. One series of samples included the top soil and surface litter, and the other series was of soil just below the surface, which was comparatively free of litter. Six-inch pots were filled with the respective soils and seeded with Thatcher wheat. The pots were kept watered and the seedlings grew at the usual fluctuating summer temperatures. They were kept in a laboratory window. The seedlings were harvested at the end of a three-week period, and notes were made regarding the degree of development of lesions at their bases. Disease rates were computed, and it was found that there were no differences that could be attributed to rotations. In every case, however, there was more disease with litter present in the soil than with soil alone. The virgin sod samples showed the lowest disease rate in the series. *H. sativum* was readily obtained from most of the lesions.

A greenhouse test was conducted to compare soil with and without litter, at a low and a high moisture level. A disease rate was determined from lesions which appeared on the seedlings growing in the soil. Here again, the soil containing litter showed the highest disease rate, regardless of moisture level. However, where litter was present, high moisture tended to depress infection. In the soil without litter, there was little difference between high and low moisture content. The low disease rate, at high moisture plus litter, was considered to be related to the increased microbial activity promoted by the litter. When, however, the soil moisture level was low with litter present, the disease rate was high, being about double the rate for soils without litter. The importance of field surface litter, consisting of stubble in most grain fields, was definitely indicated in these preliminary experiments.

In 1943, the senior author undertook a survey to determine the prevalence of *H. sativum* on stubble in wheat and barley fields. Fragments of wheat and barley straw were collected from summer-fallowed fields. It was thought that by collecting only from fallowed fields more uniformity would be obtained. All fields presented a similar history: wheat or barley crop, followed by a second crop of wheat or barley, then cultivation in the spring or early summer, climaxed by complete fallow by mid-summer. The specimens were collected in the summer. There was one important difference between the fallowed fields: some were cultivated, with most of the stubble being buried, whereas others were left with a layer of stubble and weed trash over the surface. This latter practice is common in the south and southwest as a soil drifting control. Notes were not made at the time regarding this difference, but later work indicated its possible influence on the occurrence of *H. sativum*. Collections were made around a town or village, which served as a central point. The distance between fields averaged several miles. The stubble samples were kept dry, and, later, representative specimens were incubated in the laboratory. The specimens, 100 from each field sample, were pieces of about one-inch length, including the crown. They were thoroughly washed under a tap, then placed on moist filter paper in Petri dishes. They were incubated for four days at 24° C. at the end of which sporulating growths of *H. sativum* could be determined. Eight regions were surveyed in 1943 and eleven in 1944. The results for 1944 are shown in Table 1.

TABLE 1.—PERCENTAGES OF WHEAT AND BARLEY STUBBLE SHOWING A SPORULATING GROWTH OF *Helminthosporium sativum*

Location	Field number										Average
	1	2	3	4	5	6	7	8	9	10	
	%	%	%	%	%	%	%	%	%	%	%
Swift Current	4	7	3	1	1	3	5	2	3	5	3.4
Yorkton	13	12	2	5	6	5	16	10	7	17	9.3
Pontiex	19	2	5	9	2	10	5	3	9	20	8.4
Rosetown	9	4	8	8	9	1	13	2	0	2	4.7
Tisdale	10	2	8	9	4	2	6	12	8	5	6.6
Saskatoon	0	16	2	0	2	4	2	2	9	3	4.0
Weyburn	7	2	0	4	7	5	9	1	1	3	3.8
Indian Head	22	13	8	23	3	19	15	4	3	12	12.2
Kennedy	14	8	8	4	10	2	13	15	18	1	9.3
North Battleford	3	0	7	0	4	5	6	3	0	4	3.2
Birch Hills	0	10	11	2	0	8	22	12	14	3	8.2

The most interesting feature of the tabulation is the marked fluctuations between fields. The data are too limited to bring out definite regional differences, but, so far as they go, they do not indicate a trend in that direction. The differences between fields in respect to the occurrence of *H. sativum* are very conspicuous. Fluctuations in its occurrence between fields were noticed also in 1943. A similar fluctuation in common rootrot infections, as determined by surveys, has also been observed by Sallans (16). His data, however, showed some evidence of regional differences in disease occurrence.

Collections were made of miscellaneous field litter to determine the occurrence of *H. sativum*. The collections were made in the fall near Saskatoon and included fragments of grass straws, alfalfa stems, stems of various weeds, and wheat stubble. The specimens were washed and set up in a moist chamber to incubate; *H. sativum* was obtained only from the wheat stubble. It appears therefore that cereal stubble, especially of wheat and barley, is the main site of *H. sativum* colonization.

CONIDIA ON STUBBLE AND IN SURFACE SOIL

Stubble and soil samples were collected in 1949 just after harvest and examined for *H. sativum* conidia. Pieces of stubble were examined with a binocular and the presence of conidia was recorded. Samples of surface soil from the same fields were vigorously shaken in water. After the larger soil particles had settled, the supernatant fluid was filtered, and the filter paper examined for the presence of conidia. It was realized that many, if not most, of the conidia would settle down with the soil residue. A sufficient number, however, usually remain floating to give an indication of their presence. The results are given in Table 2.

In the previous year, some soil samples were examined for the presence of *H. sativum* conidia by means of the suspension-filtration method. Conidia were quite readily found in soil collected at widely separated points, including Saskatoon, Indian Head, Raymore, Borden, Denholm, Rosetown, Shellbrook and Fielding. Conidia were difficult to find in soil from a few other locations.

TABLE 2.—NUMBER OF PIECES OF WHEAT STUBBLE BEARING CONIDIA OF *H. sativum* TOGETHER WITH RECORDS OF CONIDIA FROM SOIL SAMPLES COLLECTED AT THE SAME LOCATIONS

Location	Stubble pieces		Soil samples
	Number examined	Number bearing conidia	Conidia
Dalmeny	26	15	Few
Langham	27	17	Few
Radisson	23	10	Few
Fielding	23	10	Few
Prince	30	11	Many
Zealandia	25	2	Many

VIABILITY

The viability of the conidia found in the soil and on the stubble is of course important from a pathological standpoint. The conidia caught on the filter papers, as in the above test, were placed in Petri dishes to observe germination. It was generally found that conidia on filter papers moistened with a soil suspension would not germinate. It was thought that antibiosis set up by the soil flora inhibited germination of the conidia. Some experiments on this point will be discussed below. Almost invariably, the conidia germinated readily when picked off the soil-contaminated paper and placed on clean, moist filter paper. In some germination tests with soil solutions, mycelial fragments of *H. sativum* put out new growth, although nearby conidia failed to germinate.

Viable conidia were found in various soil samples. A sample of field soil was collected on May 13, 1948, near Saskatoon, and conidia taken from it were found to be viable. In this particular case, it seemed unlikely that these conidia were produced in the spring, as the weather had continued cool. Samples were taken from the same field on April 15, 1949, and viable conidia were found. It was, therefore, assumed that conidia remained in or on the top soil throughout the winter, and were viable. That conidia will remain viable for long periods, at least in dry soil, was indicated by testing conidia from a sample of soil which had been collected at Shellbrook in the fall and kept in dry storage all winter. The conidia were viable when tested in May. Christensen (5) found conidial viability to be maintained for long periods, even under adverse conditions.

Further information on viability was obtained by mixing conidial suspension of *H. sativum* with soil. A heavy suspension taken from cultures on potato dextrose agar was mixed with soil and the mixture placed in two Petri dishes. One dish was allowed to dry out, whereas the other was kept damp. Conidia were taken periodically for testing from both dishes. Viable conidia were still obtainable after a period of seven months. The dry soil mixture was kept and tested after twenty-three months at which time a few conidia were found to be viable. A comparable experiment was conducted in which the conidia were sprayed on to pieces of stubble kept dry in a Petri dish. When moisture was added periodically to the dish to provide conditions for incubation, the conidia on the stubble failed to germinate. If, however, they were picked off and placed on clean, moist filter paper, germination took place. This evidence supports the conclusion that the natural flora on the stubble inhibited germination.

The widespread occurrence of viable conidia in grain fields would indicate that they play an important role in primary infections in the common rootrot disease of wheat and barley.

INOCULATIONS

Information on primary infections was obtained by means of conidial suspensions in greenhouse experiments. One such pathogenicity test is outlined and discussed. Quarter-gallon crocks filled with unsterilized soil were employed. Five crocks, sown with 40 seeds each, represented the inoculated, and five similarly prepared formed the uninoculated series.

Immediately after the seed had been sown and covered, the first series was inoculated by washing a small amount of a heavy suspension of *H. sativum* over the soil surface. A day or so after the seedlings had emerged, tiny lesions were noticed on the coleoptile, at the soil surface, in the inoculated group. The lesions developed rapidly and were very distinct on the eleventh day, when the plants were harvested. The results are given in Table 3.

The results show a successful inoculation. Many experiments have been conducted in the greenhouse in which the ease of inoculation by washing a conidial suspension over the soil, before or after seedling emergence, has been demonstrated conclusively. There are, of course, many factors which may interfere with successful infections, such as soil temperature, moisture conditions, and soil type.

Only one inoculation experiment was made in the field and, although heavy infections occurred, the results were inconclusive: the check blocks became infested during a long, dry period in which surface soil blew about carrying inoculum with it.

INFECTIONS IN THE FIELD

Field surveys for root diseases were made during the summer. Particular attention was given to the appearance of lesions on wheat seedlings near the soil surface. *H. sativum* produces a rather characteristic lesion that is not likely to be confused with other infections. On one trip in June, records of lesions were kept and are shown in Table 4.

TABLE 3.—NUMBER OF WHEAT SEEDLINGS WITH OR WITHOUT LESIONS

Treatment	Number of seeds sown	Number of seedlings	
		With lesions	Without lesions
Inoculated	200	194	5
Not inoculated	200	14	176

TABLE 4.—PERCENTAGES OF WHEAT SEEDLINGS BEARING LESIONS NEAR THE SOIL SURFACE, RECORDED ON A SURVEY TRIP IN JUNE, 1949

Location	Seedlings with lesions	Location	Seedlings with lesions
	%		%
Guernsey	Trace	Viceroy	50
Fort Qu'Appelle	Trace	Limerick	40
Dafoe	100	Kincaid	80
Indian Head	100	Ponteix	65
Cupar	80	Ponteix west	40
Qu'Appelle	90	Cadillac	45
Francis	30	Masefield	60
Cedoux	50	Cadillac north	60
Trossachs	10	Elrose	10
Ogema	40	Rosetown	30

The data in Table 4 show that lesions near the soil surface are rather common. On the whole, there were more heavily infected wheat crops in the drier than in the moister areas, but there were high records also for some fields where moisture was ample. Specimens were collected from five of the fields, and pieces bearing lesions were incubated to determine the presence of *H. sativum.* The results are shown in Table 5.

TABLE 5.—NUMBER OF WHEAT SEEDLINGS BEARING LESIONS WHICH PRODUCED *Helminthosporium sativum*

Date	Locality	Number of specimens incubated	Number showing *H. sativum*
June 22	Blucher	8	3
June 22	Viscount	10	8
June 22	Dafoe	37	26
June 22	Cupar	40	27
June 24	Cadillac	24	19

The results from the incubation tests indicate that many of the lesions observed in the survey were caused by *H. sativum.*

SOIL-BORNE CONIDIA AND ANTIBIOSIS

There appears to be good evidence to show that certain microörganisms may inhibit the germination of conidia of *Helminthosporium sativum.* Such inhibition is indicated for soil solutions in the above-mentioned results on viability. The same phenomenon was observed in a study of the microflora of cereal seeds and stubble in relation to this fungus (20). Evidence of a like nature was found by Henry (9, 10) and others. It seems apparent, therefore, that conidia of *H. sativum,* in order to cause infection, must surmount the antibiosis they encounter from soil microörganisms. They must also overcome the same antibiosis before they can initiate a saprophytic development of the fungus in the soil or on crop debris.

In the opinion of the authors, the development of *H. sativum* in the field is restricted by the indigenous microflora. There is some evidence for this conclusion. When incubated stubble is examined for fungus growths, only small percentages of them, as a rule, are found to be *H. sativum.* It is thought that the sporulating colonies arise from previous infections of the culms. In earlier work, it was found difficult to inoculate unsterilized stubble pieces with conidia of *H. sativum.* An experiment conducted in the present study gives further information on this point. For the test, 500 pieces were selected from specimens collected near Saskatoon. They were washed under a tap, and 250 pieces were placed in Petri dishes fitted up as moist chambers, and allowed to incubate at 24° C. The remaining 250 were similarly set up, and inoculated with a conidial suspension of *H. sativum.* The results are shown in Table 6.

The results show that *H. sativum* was already established in about 14 per cent of the pieces of stubble. The inoculation did not greatly increase this percentage, thus indicating the difficulty of inoculating unsterilized stubble.

TABLE 6.—NUMBER OF PIECES OF WHEAT STUBBLE SHOWING *Helminthosporium sativum* AFTER INCUBATION, WITH AND WITHOUT INOCULATION, AT 24° C.

Treatment	Number of pieces	Number bearing *H. sativum*	Per cent
Incubated	250	35	14
Incubated after inoculation	250	39	15.6

The antibiotic flora found on stubble, considered here to be largely bacterial, probably arises from the soil. Presumably these bacteria are disseminated widely by wind and water. Many exploratory tests were conducted with soils and soil solutions to determine their influence on the germination of *H. sativum* conidia. As a general rule, germination was suppressed. However, when the soil solution was boiled for 10 minutes, the inhibitory effect was removed, thereby indicating the biological (bacterial) nature of the inhibition. Furthermore, non-germinating conidia in a soil smear or on incubated straws would germinate readily, as a rule, when picked off and placed on clean, moist filter paper.

Some preliminary tests were conducted with ultra-violet light and sunshine in an effort to destroy the antibiotic flora on the soil surface. In inoculation tests with *H. sativum*, treatments of the soil surface with ultra-violet light or sunshine tended to increase infection. Presumably the light treatments killed the antibiotic bacteria on the surface of the soil. The conidia on the surface, therefore, were able to germinate and cause infection without noticeable antibiotic interference. It seems reasonable to assume that, in nature, sunshine may play a similar role.

DISCUSSION

Surveys conducted over a period of years in Western Canada show the widespread occurrence of *H. sativum*, a root-disease pathogen of wheat and barley. A review of the literature indicates a similar prevalence in the extensive grain growing areas of the United States. Recent surveys in Saskatchewan show wide fluctuations in the prevalence of the pathogen or the disease from field to field. This feature of the disease has never been satisfactorily explained, although common rootrot has been studied by many researchers. Moreover, the onset of the disease, especially in regard to primary lesions, has not been explored thoroughly.

In the present studies, conidia of *H. sativum* were found to be abundant both on the stubble and in the surface soil of some fields. Many of the conidia were viable, even when taken from soil samples early in the spring. It is apparent, therefore, that they can survive the winter. Laboratory experiments demonstrated, furthermore, that conidia would remain viable for long periods in soil. It is believed that conidia, in or on the surface soil or on stubble, are a source of primary infections in the crop. Surveys in June revealed the common occurrence of infections in the crops, presumably from this source. Greenhouse experiments show that wheat or barley seedlings were readily inoculated by washing a conidial suspension over the soil surface. Inoculations of this type could be made successfully either before or after seedling emergence.

It should be mentioned that the infection of seedlings near the soil surface permits the pathogen to enter the crown node or adjacent tissues. Hynes (11), Sallans and Ledingham (15), and Greaney (7) found that invasions at this point, with the subsequent destruction of crown and subcrown internode tissues, tiller buds, and root initials, may cause serious injury under conditions favourable for disease development.

The conclusion that an abundance of *H. sativum* conidia may be found in or on the surface of the soil, makes it possible to formulate a seasonal cycle respecting disease incidence. Briefly, such a cycle may be outlined as follows: (1) conidia present in or on the surface soil cause primary infections; in time, new conidia are produced; and new infections occur throughout the crop season; (2) after harvest, conidial production continues on the stubble of plants which bore lesions. Thus, throughout the summer and fall, conidia are deposited on the soil, to be washed about by fall, winter, and spring rains. These conidia serve as inoculum for the next crop.

Conidia may also be moved about by high winds. It is thought, however, that they are not well adapted for air dissemination. They have rarely been present on spore-trap slides used in rust epidemiology studies in Western Canada.

It has been shown in the present work, in previous studies by Simmonds (20), and in investigations by others that *H. sativum* may be suppressed by other microörganisms. It would be expected, therefore, that conidia of this fungus on the soil surface would be exposed to rather active antibiosis by the indigenous flora. This antibiosis would fluctuate with changes in moisture, temperature, and sunshine. There would also be considerable variation in the number and kinds of microörganisms (bacteria) involved. Some support for the assumption of an antibiosis inhibitory to *H. sativum* was obtained by exposing the soil surface to ultra-violet light or sunshine. Such exposures, in inoculation tests, gave higher infections of seedlings than in comparable but shaded soils. The results of these tests support the hypothesis that the light treatments killed or inactivated the antibiotic bacteria on the soil surface, thus allowing the free development of *H. sativum*. It is believed that such phenomena could take place in nature.

The antibiosis in the top soil, which may inactivate conidia, is no doubt influenced by other environmental conditions brought about by cultivation, rotation, fertilizers, and so forth. Fluctuations, such as do occur, in the incidence of the disease from field to field and district to district might, therefore, be expected.

If conidia on the surface of the soil are as important as we believe they are in causing primary infections, then the surface soil becomes a pivotal point in devising methods of control. In this regard, attention should be directed towards ways and means, perhaps by cultural methods and rotations, whereby the antibiosis at the soil surface could be enhanced. Perhaps, also, antibiotic substances could be applied directly to the soil surface. On the other hand, it might be feasible in some instances to apply fungicides to kill surface-borne conidia.

SUMMARY

Survey records on the occurrence of *Helminthosporium sativum* in Western Canada are discussed. Wide fluctuations in incidence between fields was found.

It is shown that *H. sativum* exists in great abundance in some fields as conidia on the stubble or in the surface soil. These conidia retain their viability for long periods and frequently over winter. Experiments conducted in the greenhouse as well as field surveys indicate that such conidia are important in causing primary infection on wheat seedlings.

A seasonal cycle pertaining to the occurrence of the pathogen and the appearance of the disease is outlined.

Tests were conducted which showed that the germination of conidia of *H. sativum* was suppressed by the microflora on stubble or in the top soil. This microflora was considered to be largely bacterial. The influence of ultra-violet light and sunshine in retarding or killing the bacteria on the surface of the soil, without injuring the conidia, was indicated by experiments.

The possibility of employing the knowledge regarding conidia on or in the surface soil and information on the antibiosis of the soil flora in designing control methods is discussed.

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SPRINKLER IRRIGATION EXPERIENCE IN BRITISH COLUMBIA ORCHARDS[1]

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HISTORY OF SPRINKLER IRRIGATION IN BRITISH COLUMBIA

Sprinkler irrigation of orchards was started commercially in British Columbia about 1930. During the next five years, sprinkler installations were made in most of the orchards in the Creston district, and in a few orchards in the Penticton district. These early installations consisted for the most part of underground iron pipe, above-ground taps, garden hoses, and low-pressure undertree sprinklers of the butterfly type. This type of installation in some cases has been replaced recently by the portable pipe type; but as far as the author is aware, all of these orchards are still being irrigated by the sprinkler method.

In 1945 the portable pipe and quick coupler type of installation was first used in British Columbia orchards. Each year since then, many growers in the southern interior of the province have changed over from the furrow or rill method of irrigation to the sprinkler method, until at the present time approximately 30 per cent of the orchards in the irrigated districts are irrigated with sprinklers. The total area of orchards now under sprinklers is over 11,000 acres. Only one case has been reported to the author where a grower has changed from furrow irrigation to sprinkler irrigation and has then changed back to the furrow method. Almost all of the orchards are irrigated with low-pressure, undertree sprinklers. High-pressure, overtree sprinklers have been installed in only three orchards.

The usual type of sprinkler installation in British Columbia orchards consists of a screening box or tank to remove coarse dirt from the water; a pump and motor (where these are needed); an underground mainline steel pipe through the centre of the orchard; hydrants at intervals of 90 to 150 feet along the mainline; and portable sprinkler lines at right angles to the mainline, each 300 to 600 feet in length. A portable line usually consists of 2-inch aluminum tubing, in lengths of 20 to 30 feet, these lengths being held together with quick couplers. Rising from each coupler is a short ½-inch pipe topped by a rotary sprinkler. Each portable line is usually run for 12 hours along a tree panel, and is then moved to the next panel for the next 12 hours. Each panel is thus covered at intervals of 7 days to 30 days, depending chiefly on the type of soil.

After an initial study of sprinkler irrigation at this Station, recommendations were made to growers of tree fruits in 1946 to consider the sprinkler method favourably under the following circumstances:

1. Where soil erosion is in evidence.
2. Where the soil is sandy.
3. Where the contour is irregular.
4. Where the soil is saline.

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No doubt the growers have been strongly influenced by these recommendations. They have, however, been influenced also by various other considerations. For example, some growers prefer sprinklers because they save water; others because they save labour. Some growers stress the ease with which they can grow a good cover crop under sprinklers, others the fact that couch (quack) grass does not worry them so much. Many reasons are given for the change-over by individual growers. It is worthy of note, however, that interest in the sprinkler method has been keenest where the contour of the land is steep or irregular and where the soil is sandy.

It is the major purpose of this paper to outline in brief the experience of tree fruit growers and the experience of the Experimental Station at Summerland with sprinkler irrigation during the past five years. To this end, conferences have been held with growers, irrigation district officials and sprinkler equipment agencies throughout the southern interior of the province; and in addition, most of the types of equipment used by growers have been placed under actual use tests at this Station.

In the past, the standard method of orchard irrigation in British Columbia has been the furrow or rill method. Due to steep and irregular contours, light soils, high cost of water and other factors, flood irrigation has not proved feasible for orchard irrigation. In assessing the value of sprinkler irrigation, therefore, it is only possible in this area to make direct comparison with furrow irrigation.

Favourable Experiences with Sprinkler Irrigation

The first beneficial effect observed from the use of sprinklers, as compared with furrow irrigation, has been a reduction in soil erosion. A high percentage of the orchards now under sprinklers are situated on slopes. Prior to the use of sprinklers, erosion had been serious in many of these orchards, especially where the soil was sandy. Each year considerable washing of soil occurred along the sides and bottoms of the furrows, and in some of the orchards examined the original surface soil had been completely lost after only 30 to 40 years of irrigation. An examination of these same orchards, made following the installation of sprinkler irrigation, has revealed only slight if any surface erosion from sprinkler irrigation. Where the grower has a sod grass or sod legume cover crop and does not cultivate during the growing season, erosion caused by sprinkling is usually too small to measure.

Under furrow irrigation, it is often difficult to obtain a good catch of the cover crop, especially between the furrows; and it is still more difficult to maintain it once it has caught. This is especially true between the furrows in sandy soils. Under sprinkler irrigation, most growers have been able to obtain an excellent catch of the cover crop, and to maintain it in a vigorous condition without difficulty. The standard practice is to plant a sod cover crop such as Kentucky blue grass or ladino clover, and to mow it, rather than cultivate it. Mowing helps to maintain the cover crop, and is cheaper than cultivation; in addition, it does not induce soil erosion as does cultivation. Mowing is much easier to perform under sprinkler irrigation than under furrow irrigation.

Most growers have expressed themselves as being glad to be rid of the furrows. They find them expensive to make and to maintain, especially in a sod grass cover crop such as couch grass. Moreover, with furrows present the ground is more uneven and orchard operations are more difficult to perform. Some growers report distinctly less bruising of the fruit during hauling, when furrows are absent.

An examination of the soil in a large number of orchards indicates that irrigation water is being applied to the soil much less uniformly by the furrow method than by the sprinkler method. This is especially true in sandy soils, where the soil is seldom wetted properly between the furrows. Even in clay soils, however, dry spots are frequently found between the furrows; for example, along the tree rows where the furrows are usually wider apart

Most growers have reported a distinct saving in water on changing from furrows to sprinklers. This saving usually amounts to about 10 per cent with heavy soils and about 35 per cent with sandy soils, although figures from zero to over 50 per cent have been reported. In some cases, the saving on sandy soil has been little, if any; and in such cases investigation usually reveals that under furrow irrigation there had not been sufficient water to maintain adequate soil moisture. In some such cases, however, the sprinkler system has been poorly engineered and the grower is using far more water than he should. Irrigation districts have reported savings averaging around 20 to 30 per cent, depending chiefly on the kind of soil in the district. Careful comparison made in sandy loam soils at the Experimental Station at Summerland and at the Substation at Kelowna, resulted in savings of about 30 per cent at each place over a period of three years. Some of this saving resulted from a reduction in amount of water lost into the deep subsoil below the furrows, some from elimination of the tailings water that runs off the lower ends of the furrows.

Preliminary investigations on water requirements under sprinkler irrigation have led to tentative recommendations for the southern part of the Okanagan Valley. These are summarized in Table 1. They apply to mature orchards with permanent sod legume or sod grass cover crops. The recommendations vary in accordance with the length of interval that can safely be allowed between irrigations, i.e., from as little as five days in a shallow sand or gravelly soil to as much as one month in a deep silt or clay loam.

Reports from all districts where sprinklers are in common use reveal that difficulties caused by tailings water and seepage water have been greatly reduced. In some orchards it has been found very difficult to control tailings water, and in many cases it has collected and run down the municipal roadway, over a bank, or through the neighbour's orchard, causing serious damage in its path. Seepage water has emerged lower down the slope or on the valley bottom and has induced both excess water and excessive salinity at that point. In some cases serious slides have been produced on the hillsides, due to weakening of the banks by seepage water. Complaints of such difficulties are seldom heard where sprinkling has become the general method of irrigation. Periodical examinations of drainage systems under a number of orchards have revealed a marked reduction in the flow of drainage water following the installation of sprinkler systems.

TABLE 1.—SPRINKLER IRRIGATION RECOMMENDATIONS FOR MATURE ORCHARDS IN SOUTHERN OKANAGAN VALLEY

Safe interval	Amount per application	Amount per month	Rate of flow per acre	Suitable length of irrigation	Rate of application per hour
days	inches	inches	g.p.m.*	hours	inches
5	2	12	6.3	8	0.25
7	2½	10	5.2	8	0.31
10	3	9	4.7	12	0.25
15	4	8	4.2	12	0.33
21†	5	7	3.7	12	0.42
	5	7	3.7	24	0.21
30	6	6	3.1	24	0.25

* Imperial gallons per minute.
† Two alternative rates of application are suggested, depending on the soil permeability.

In every case reported to the author, growers have claimed a saving in labour on changing to sprinklers. The saving claimed in the daily work of irrigation has varied widely, but usually falls within the range of 40 to 60 per cent. These high figures are possibly due in large measure to the fact that many of the orchards that were changed over to sprinklers were poorly adapted to furrow irrigation in the first place, so that labour costs had been quite high. The operator with sprinklers usually changes his portable lines twice a day, and spends the rest of the day conducting other orchard operations; whereas the man using furrows may have to spend most of the day adjusting the flow of water and cleaning out plugged furrows. In addition to this routine saving in time is the saving resulting from not having to make furrows once or twice each year and connect them up to the flumes by hand.

No experiments have been conducted in British Columbia to compare the effects of furrow irrigation and sprinkler irrigation on tree yield. The only yield-comparison records available are those provided by growers who have changed from furrows to sprinklers. These records, however, cannot always be relied upon, since yield comparisons are usually made in different years. Some growers report marked increases in yield following installation of sprinklers, especially with peaches and apricots in sandy soil. Observational evidence indicates that these yield increases are often due in large part to the use of sprinklers; but there is no experimental proof that such is the case.

Unfavourable Experiences with Sprinkler Irrigation

When sprinkler irrigation assumed importance in British Columbia orchards, it was feared that spray from the sprinklers would wet the leaves and fruit, and because of this would aggravate diseases or insect pests or possibly wash off the spray materials. On this account, it was recommended that undertree sprinklers with a low trajectory be used. Actually, the deleterious effects induced by wetting of the leaves and fruit have not proved to be as serious or as widespread as was feared. Numerous cases have been reported, however, where some damage appears to have been caused by sprinklers which have thrown their spray too high into the trees. Brown rot of peaches appears to have been somewhat worse in such cases; also fire blight of pears. *Phytophthora cactorum*, which causes crown rot of apples, has caused rotting of apricot, peach and pear fruits. Reports on apple scab are somewhat conflicting; so also are the reports on splitting of cherries and apricots. There appears to be no doubt that under certain circumstances cherries are split more when wetted by sprinklers. Even though such cases of damage have not been widespread, overtree sprinklers are still not being recommended for general use.

Over-irrigation has been indulged in by a few growers with sprinkler systems. This has not only wasted water, but had in some cases caused actual damage. A case in point was where the grower has a heavy soil with poor drainage. Under furrow irrigation he had wasted a large percentage of the water over the ends of the furrows. When he changed to sprinklers, he continued to use his full quota of water and thus applied too much and nearly killed his trees. Such over-use of water is sometimes caused by poor engineering of the sprinkler system, sometimes by lack of understanding on the part of the grower.

Most growers have installed adequate screening boxes or drums at their intakes, to keep coarse dirt out of their sprinkler lines. Where this has not been done the sprinklers have plugged up and distribution of water has not been uniform. In some districts, however, difficulties have been encountered from fine dirt in the water, that stays in suspension and quickly wears out the sprinkler heads. The sprinklers may not turn at uniform rates in such a case, and replacement parts are needed frequently.

As already noted, it has become customary to grow sod grass or sod legume cover crops in sprinkled orchards. This has no doubt constituted one of the factors encouraging the present plague of mice. Although the mice are prevalent in clean-cultivated orchards, they are still worse in sod orchards. Present recommendations in British Columbia are for extermination with poison bait, as they have become a menace under any cultural conditions.

Some growers have complained that sprinkling brings the roots of the trees closer to the surface; and investigation has revealed that in many cases this is true. However, this of itself does not appear to constitute an undesirable effect. If the roots were to stop growing deep into the soil, then the situation would be more serious; but under normal soil conditions such an effect has not been found. No evidence has yet been obtained to indicate that the trees have suffered to any extent from having shallow roots as well as deep roots. Rooting in the fertile upper layers of soil may actually be an advantage.

Special Problems Due to Sprinkler Irrigation

When any new procedure is adopted by farmers, special problems arise that require special investigation. This has held true in the case of sprinkler irrigation in British Columbia. Some of the problems have been solved and some have not. A few of them will be mentioned in case they should prove of interest elsewhere.

Early in the investigational work on sprinklers, it was found that best distribution of the water was obtained when the sprinklers were placed in the centres of the tree squares. In order to accomplish this, the grower

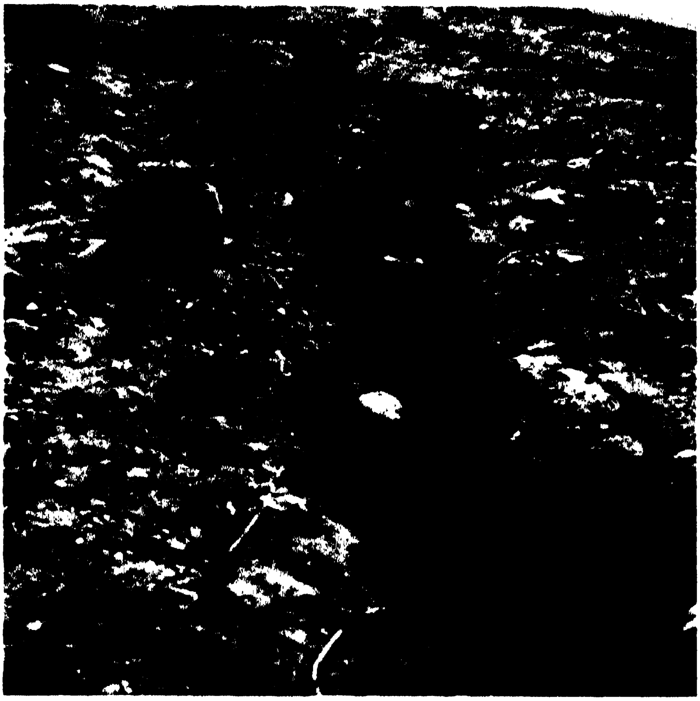

FIGURE 2. Under furrow irrigation, soil erosion has proved to be much more severe than under sprinkler irrigation.

FIGURE 3. Under furrow irrigation, tailings water frequently collects and causes damage to roadways or to the neighbour's orchard.

Figure 4. A low-pressure type of sprinkler is usually used in orchards. It operates at optimum pressures of 20 to 30 pounds per square inch.

Figure 5. Poor engineering of a sprinkler system sometimes results in too rapid a rate of application of the water. This is more commonly found on heavy soils.

needed portable pipe in the same lengths as the tree spacing. At first the only length stocked by dealers was 20 feet. They are now stocking also 25- and 30-foot lengths, and other lengths can be obtained on special order. These "special orders" usually cost more. A common difficulty encountered is where a grower has two or more distances of planting in the same orchard, so that it would not be feasible for him to place every sprinkler in the middle of a tree square. On the whole, this problem does not appear to be a serious one at present.

Somewhat more serious is the problem of obtaining good equipment, that will give adequate service for a reasonable length of time. Second-hand pipe has been sold for underground use that has leaked badly when first used. The new 3- and 4-inch steel pipe in common use for the main lines is seldom heavier than 16-gauge. Sprinklers and couplers have been sold that were not suitable for orchard use. Such weaknesses have been due in part to lack of knowledge and in part to keen competition. Dealers find that in too many cases they must give low quotations on rather poor material if they are to obtain an order. In some cases, of course, suitable materials have not been available.

A similar difficulty has been encountered in the engineering of the sprinkler installations. Most growers know little about it until they have had some experience with sprinkling, and they cannot engineer their own installations. The equipment dealers on the whole have been doing a good job for them. Numerous cases have been encountered, however, where serious mistakes have been made. For example, systems have been designed that apply water much too rapidly to heavy soils; or that apply much more water than needed; or that use too high or too low a pressure, or that get around at too long an interval on sandy soils, or that place the sprinklers too far apart for adequate distribution of the water. Some of the mistakes made can be classified as engineering errors, some perhaps as due to lack of knowledge of irrigation requirements; and in some cases they may have been due to the dealer's desire to make low quotations on the equipment. The technical workers and the dealers are now co-operating in an effort to improve the situation with regard to equipment and engineering, and rapid strides have already been made toward this end.

As already noted, many of the orchards are planted on hillsides. It has been found difficult in many such orchards to operate all of the sprinklers within a reasonable pressure range. It has been recommended by this Station that the greatest and least delivery pressures at the sprinklers should not differ by more than 20 per cent of the highest pressure in any one orchard. To accomplish this, the portable lines are run on the contour wherever feasible, and the pressure in each line is regulated by the valve opening on to it. In some cases, however, a portable line will run uphill or downhill, thus causing wide variations in pressure. Various methods are in use for overcoming this difficulty, such as partial stops or valves along the portable line, or different sized sprinkler nozzles; or better still, a small valve under each sprinkler. Such methods are better than nothing, but are not entirely satisfactory.

Most of the fruit growers in the irrigated areas are grouped together into co-operative organizations called "Irrigation Districts". The districts impound the water and deliver it to each orchard, sometimes in flumes or ditches, sometimes in pipes. It has been customary under furrow irrigation to measure the water to each orchard over an open weir. This is still done when the water is delivered in a flume to a sprinkled orchard. When it is delivered in a pipe, however, the grower does not wish to lose the pressure, and he objects to having this water measured over an open weir.

Among the methods being tried or considered for measuring the water without losing pressure are the following: (1) A meter large enough to handle the full flow. This is very expensive. (2) Tapping a small known proportion of the flow and passing this through a meter. (3) Using one meter to measure the proper flow at each orchard in turn, and locking the district valve at that point. A second valve is then required by the grower. (4) Inspecting each orchard periodically during the irrigation season to ensure that each grower is using the number of sprinklers, nozzle size and pressure that are required for the rate of water delivery allowed. So far, this last method has proved the most popular, in spite of its weaknesses. Little serious difficulty has yet been encountered in over-use of water by growers with sprinklers, as most of them have been using less water than districts allow for furrow irrigation.

Comparative Costs of Sprinkler and Furrow Irrigation

A question asked more frequently than any other in connection with sprinkler irrigation is: "How do the costs of this method of irrigation compare with those of other methods?" It has not been found possible to compare costs of sprinkler irrigation with those of flood irrigation in British Columbia orchards, but reasonably good comparisons have been obtained between sprinkler irrigation costs and furrow irrigation costs.

Costs of irrigation, as far as the orchardist is concerned, can be divided generally into the following categories:—(1) Cost of water; (2) Cost of equipment; (3) Annual operating costs (other than interest and depreciation), and (4) Effects on costs of other orchard operations.

1. COST OF WATER. In some Irrigation Districts, water is paid for by the grower in two ways—first, as a tax on the per-acre basis, to pay for the costs of dams and delivery systems; and second, as a toll based on the amount of water used, to pay for current District operating expenses. Where the grower requires less water because he has changed to sprinklers, his toll charges are somewhat reduced. This has usually had only a minor effect on total costs.

In some Irrigation Districts, water is paid for on the per-acre basis only. In such cases the individual grower receives no direct reduction in water charges when he changes to sprinklers. When enough growers have changed to sprinklers, however, the District may find it has excess water on its hands, more land could be allowed to come under irrigation, and the per-acre charges could then be reduced somewhat; or more likely, these charges would not be increased as much as would otherwise be the case with a general increase in costs of materials and labour. It is very difficult to assess accurately such savings in costs.

2. COST OF EQUIPMENT. The cost of equipment and the cost of its installation should be considered together. These costs are comparatively high in British Columbia orchards, with either sprinkler or furrow irrigation.

This is due in part to the high costs of materials and equipment, and in part to such factors as small size of orchard, rough nature of much of the terrain, and sandy nature of much of the soil. These factors vary so widely that costs likewise vary widely. In the following discussion, an average type of 10-20 acre orchard will be used as a basis for cost comparisons.

A number of reasonably accurate records have been obtained on the costs of installing metal and cement fluming in orchards. At present costs for materials and labour, eight-inch metal or cement fluming costs approximately 38¢. per foot installed, and ten-inch 46¢. In addition, there are costs for distribution boxes, screening boxes, etc. Such a system costs at least $70 per acre installed, in the average orchard.

The cost of installing a sprinkler system is usually still higher. In this case, most of the initial cost is for equipment, little of it for labour. An examination of growers' costs reveals costs varying all the way from $40 to $200 per acre. Where the grower has water delivered to his orchard under pressure, the average cost appears to be about $100 per acre. This figure includes the costs of main line pipe and its installation, portable pipe, couplers, sprinklers, screening tank, etc. However, where the grower needs to develop pressure for sprinkling, either with a pump and motor or with a pipe leading to a source of supply of water at a higher elevation, his costs mount. In such cases the average cost appears to be about $130 per acre.

In comparing the costs of installing flume systems and sprinkler systems, it is assumed that one or the other is actually needed. It sometimes happens, however, that a grower who already has a satisfactory fluming system decides that for reasons other than cost he wishes to install a sprinkler system. In such a case the above comparison will not apply.

3. Operating Costs. The chief operating costs are labour, tractor, sundry repairs, and electricity. A large number of records obtained in grower orchards and on the Experimental Station indicate that usually these operating costs are substantially lower with sprinkler irrigation than with furrow irrigation. This is due in large measure to higher labour costs by the furrow method. Each year the grower cultivates, makes new furrows in the spring with tractor and ditcher, connects them to the flumes by hand, and cleans them out in midsummer. The uneven contours encountered in many orchards make it difficult for an irrigator to handle more than 25 or 30 acres working full time during the irrigation season. In comparison with this, a sprinkler operator can usually handle the water with less than half the daily labour costs of those with furrows, and no costs for making or maintaining furrows. On the average, this saving with sprinklers amounts to at least ten labour hours per acre during the season, in addition to tractor hours.

Where the sprinkler system requires the development of pressure with a pump and motor, the costs of electricity are substantial. With the low pressure sprinklers, such as are used in orchards, pumping heads of 50 to 70 feet are usually required. With electricity charged for at the rate of $4.50 per horse power per month, the annual costs for this item usually run about $3.50 to $4.00 per acre for the season. Where the water is pumped from a lake or river, the cost of electricity is often no higher for sprinkler irrigation than for furrow irrigation, because of the need for less water by the former method.

4. EFFECTS ON OTHER COSTS. No attempt has been made to assess the effects of sprinkler irrigation on other costs. Growers who have changed to sprinklers claim a reduction in cost of handling the cover crop. They find mowing to be cheaper than cultivating. They also claim that the elimination of furrows reduces slightly the cost of each orchard operation such as spraying, thinning, picking and hauling, and that the over-all reduction for these operations is substantial.

It will be seen from the above discussion that only in certain items of cost has it been possible to compare sprinkler irrigation and furrow irrigation with any degree of accuracy. It is of interest to note how these particular items balance against one another.

The only satisfactory way of making a final cost comparison is on the basis of annual operating costs, including interest and depreciation. It should be sufficient to consider only the estimated *differences* between the two methods. The average difference in cost of equipment and installation is possibly $30 per acre where water is delivered under pressure, and about $60 per acre where a pump and motor are required to develop pressure for sprinkling. If 12½ per cent is allowed annually for depreciation and interest, to cover the additional cost of sprinkler equipment at $30, this would amount to an added $3.75 per acre where water is delivered under pressure, less an estimated saving of $7.50 per acre for labour and tractor power or an apparent net saving of $3.75 per acre. Where a pump is required and the additional investment amounts to $60 per acre, the added overhead would be $7.50 per acre. This would be offset by the annual saving of around $7.50 per acre for labour and tractor. However, there would still be the added cost for electric power to the pump of about $4.00 per acre as compared with furrow irrigation.

It should be clearly understood that the above estimates represent an average for 10- to 20-acre orchards only and that there are wide variations from these figures. As already noted, both costs of irrigation equipment and hours of irrigation labour vary widely from orchard to orchard. Every orchard should, therefore, be considered as an individual problem in so far as the design and cost of the irrigation system are concerned and the probable savings in labour and expense with any particular method of irrigation.

It should be emphasized again that these comparisons of the sprinkler method and the furrow method apply only to those conditions encountered in the orchard districts of the southern interior of British Columbia. They do not necessarily apply to other crops, nor to tree fruits in other areas.

SUMMARY

Sprinkler irrigation of orchards was started in British Columbia about 1930, but did not become popular with growers until portable pipe was placed on sale in 1945. About 30 per cent of the orchards are now irrigated by sprinkling. Low-pressure, undertree rotary sprinklers are used almost entirely.

In comparison with furrow irrigation, the sprinkler method has brought about a marked reduction in soil erosion. It has also induced better growth

of cover crops, made orchard operations easier to perform, provided more satisfactory soil moisture conditions, saved water, saved labour, and reduced difficulties from seepage water.

On the other hand, some slight difficulty has been encountered from increased brown rot of peaches, fire blight of pears, splitting of cherries and apricots. Some growers using sprinklers are over-irrigating, or are not applying the water uniformly enough, or are not screening the water adequately. Some sprinkler systems have been poorly engineered, or contain equipment poor in quality. Irrigation districts have encountered some difficulty in measuring water under pressure.

Cost comparisons of furrow irrigation and sprinkler irrigation indicate that in Okanagan Valley orchards the former is usually cheaper to install and the latter cheaper to operate. When all items of cost are taken into consideration, they appear to average approximately the same by the two methods.

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STUDIES ON THE COMPETITION OF CRESTED WHEATGRASS WITH PERENNIAL NATIVE SPECIES[1]

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INTRODUCTION

Crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.) has been used extensively during the past fifteen years in the drier parts of Saskatchewan and Alberta for regrassing abandoned farm lands and depleted range pastures. In many sections it is the only grass generally recommended for these purposes. In view of this recognized economic importance of the species, it was decided to obtain more exact information on its reaction to competition from established stands of perennial native species. For this purpose, permanent quadrats were established at Mortlach, Saskatchewan, in 1938 on selected sites. These quadrats were recharted in 1940 and another group initiated at Webb, Saskatchewan, the same year. Unfortunately, reductions of staff and equipment during the war years, and for some time afterwards, prevented further study of the quadrats until 1948. At this time, recharting was undertaken and the results have been summarized for presentation in this paper.

LITERATURE REVIEW

Hubbard (5) reported data extending over a 20-year period from 1928 to 1948. He found that crested wheatgrass controlled annual weeds even when seeded in rows 3 feet apart. However, it did not invade adjacent stands of native sod dominated by blue grama grass (*Bouteloua gracilis* (H.B.K.) Lag.) and common speargrass (*Stipa comata* Trin. and Rupr.). On the other hand, these grasses invaded established stands of crested wheatgrass and the invasion was considerably increased when the area was heavily spring grazed as compared to complete protection from grazing. Crested wheatgrass seeded into sod was not successful unless the native species had been first depleted by drought or cultivation. However, once crested wheatgrass was established in native sod it increased in basal area, although the increase was small unless there was protection from grazing. Allred (1) commented on studies and observations covering the Northern Great Plains. He noted that crested wheatgrass migrates successfully into communities of annual weeds and that minor migrations into open stands of blue grama grass have taken place. He also reported that a thrifty stand of crested wheatgrass had become established in a 25 per cent stand of saltgrass (*Distichlis stricta* (Torr.) Rydb.). However, in regard to climax communities, Allred states: "Although such cases have been reported, I have never seen a situation where crested wheatgrass has migrated into a climax stand of grasses or even produced competitive stands when it has been drilled into them." Blaisdell (2) studied the development of crested wheatgrass and other grasses when they were seeded prior to, concurrently with, and subsequent to sagebush (*Artemisia tridentata* Nutt.) and (*A. tripartita* Rydb.). His results showed that sagebush did not invade an established stand of crested wheatgrass but that when seeded concurrently with crested wheatgrass the sagebush was suppressed at first but later gained a prominent position in the stand. Sagebush stands two years old or older were not greatly affected by crested wheatgrass seeded into them and the older the stands of sagebush the more difficult it was for the crested wheatgrass to become established. The sagebush-grass mixtures, however, gave much higher total yields than sagebush alone. Pavlychenko (7) studied competition between crested wheatgrass and perennial weedy species after the grass had been seeded into well established stands of the weeds. He reported that sow thistle (*Sonchus arvensis* L.) was completely suppressed in three years, Canada thistle (*Circium arvense* (L.) Scop.) in five years, and that quackgrass (*Agropyron repens* L.) was almost gone in four years. Poverty weed (*Iva axillaris* Pursh), leafy spurge (*Euphorbia Esula* L.), field bindweed (*Convolvulus arvensis* L.), and hoary cress (*Cardaria Draba* (L.) Desv.) had not disappeared after four years of competition but only weak plants were left. Hull and Stewart (6) found that crested wheatgrass survived and spread in stands of wheatgrass (*Bromus tectorum* L.). Reitz *et al.* (9) recommended crested wheatgrass for reseeding abandoned farm lands occupied by Russian thistle (*Salsola pestifer* A. Nels.) and tumbling mustard (*Sisymbrium altissimum* L.), but obtained only scattered stands when it was seeded into native sod. They concluded that the native species must be more or less completely subdued before successful stands of crested wheatgrass can become established. Westover and Rogler (11) state that weeds are not likely to cause trouble in closely drilled stands of crested wheatgrass. Peake and Chester (8) and Clarke and Heinrichs (4) found crested wheatgrass the most suitable species for reseeding abandoned farm lands. They also reported that it replaced pasture sage (*Artemisia frigida* Willd.) in three years after seeding.

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METHODS OF STUDY

The permanent square meter chart quadrat was used almost exclusively in the studies. The only exception was a permanent belt transect established on the Mortlach area. In all cases the methods of locating and charting the quadrats and transect were similar to those described by Weaver and Clements (10). The basal area of each plant was taken to be the area occupied by its vertical projection at a point about 2 inches above the ground surface. Actual areas were estimated from the charts by the use of transparent squared paper. The quadrats on the Mortlach area were numbered from 1 to 10 and those on the Webb area from 11 to 24.

DESCRIPTION OF STUDY AREAS

Mortlach, Saskatchewan

The Mortlach study area was located on Section 32, Township 17, Range 1, west of the 3rd meridian. The actual field studied had been seeded to Fairway crested wheatgrass in the fall of 1936 at a rate of 10 pounds per acre in rows 6 inches apart. At the time of seeding the field was abandoned farm-land. The date of last cultivation was unknown but it was apparent that the field had not been cropped for several years. Part of the field was in the annual weed stage but portions of it, particularly around the edges, were dominated by native perennial species. An excellent stand of crested wheatgrass had become established wherever it was seeded among the annual species. Among perennial species the stand was thinner but was considered sufficient to eventually dominate if it was successful in competing with the native species.

At no time during the period of study was the area grazed except during the fall and winter after all vegetation had matured. In occasional years the crop was harvested for hay.

The soil texture of the Mortlach area is a sandy loam and is well drained except for a small dry slough or shallow depression. Quadrat 1 was located in the centre of the slough, quadrat 2 at the edge, and quadrats 3, 4, 5, and 6 about half-way up the slope. The transect extended from high ground down to the edge of the depression. Quadrats 7, 8, 9, and 10 were located on surrounding high ground. Representative samples of soil from the 0-6 inch layer and the 6-12 inch layer were analysed for soluble salt content. The results are presented in Table 1.

The analyses shown in Table 1 are typical of similar locations in Saskatchewan. The soluble salts in depressions leach downwards and a later lateral movement results in their deposition by capillary action near the surface of the slope. Consequently, these slopes tend to be dominated by characteristically saline species such as alkali grass (*Distichlis stricta* (Torr.) Rydb.) and alkali cordgrass (*Spartina gracilis* Trin.).

The nearest detailed climatic records were those of the Moose Jaw Station about 25 miles east of the study area. During the 10-year period 1939-1948, the mean maximum temperature for the months May-October inclusive was 68.1° F. The corresponding mean rainfall was 11.3 inches. Temperature records varied between a low of 65.4° F. in 1945 to a high of 70.5° F. in 1940. Rainfall data ranged from 8.1 inches in 1948 to 18.4 inches in 1942. It is believed that similar general climatic conditions prevailed at Mortlach. The heavy rainfall and low temperatures (mean of 65.8° F.) in 1942 probably caused flooding. The effect of this on the species composition of quadrats 1 and 2 will be discussed later.

TABLE 1.—SOLUBLE SALT CONTENT OF SOIL SAMPLES TAKEN FROM THREE DIFFERENT LOCATIONS AT MORTLACH

Location of sample	Depth of soil	Soluble salt content in per cent					
		$CASO_4$	$MgSO_4$	NA_2SO_4	$NaHCO_3$	NaCl	Total
	(in.)						
Side hill near quadrats 3 and 4	0–6	0.061	0.025	—	0.049	0.020	0.155
	6–12	0.343	0.658	0.694	0.015	0.058	1.768
Highland near quadrat 9	0–6	0.017	0.020	—	0.025	0.010	0.072
	6–12	0.020	0.020	—	0.018	0.010	0.068
Slough near quadrat 1	0–6	0.024	0.010	—	0.010	0.012	0.056
	6–12	0.020	0.015	—	0.013	0.010	0.058

Webb, Saskatchewan

The Webb study area was located on Section 2, Township 15, Range 16, west of the 3rd meridian. The field on which the quadrats were situated was seeded to Fairway crested wheatgrass in April, 1939 and is in a community pasture that was grazed moderately to heavily every year from 1940 to 1948. Drainage is good throughout and the soil texture is a fine sandy loam to sandy loam. Quadrats 11 to 15, inclusive, represented abandoned farm-land and flat topography. The remainder (Numbers 16-24, inclusive) were located on the south and west slopes of a high knoll where the soil had never been broken although at the time of seeding the native species were in a badly depleted condition caused by overgrazing.

As at Mortlach, no detailed climatic data were recorded adjacent to the study area. However, records were available at Swift Current about 20 miles northeast of the project. The mean maximum temperature at Swift Current from May to October, inclusive, for the 8-year period 1941-1948 was 65.9° F. The corresponding mean season rainfall was 11.3 inches. Seasonal temperature means varied from 63.2° F. in 1942 and 1945 to 68.8° F. in 1944. Seasonal rainfall ranged from 8.6 inches in 1948 to 17.9 inches in 1942. There was a remarkably close agreement of temperature and rainfall data from Moose Jaw and Swift Current. This suggests that these two stations, and hence the Mortlach and Webb study areas, have similar climatic conditions.

SUMMARY OF COMPETITION STUDIES

Mortlach Area

Belt Transect.—The belt transect was charted in study competition under varying soil moisture and soil salinity conditions. Each section of the transect was one meter long and three decimeters wide—all 9 sections forming a continuous transect from the dry upland down to the edge of the slough. Sections A, B, and C represent a dry situation at the top of the slope, sections D, E, and F moderately good moisture conditions with relatively high salt content (see Table 1) midway down the slope, and sections G, H, and I favourable moisture levels near the edge of the slough. Since these three general conditions seemed to form natural divisions the data were grouped according to conditions of soil moisture and soil salinity and are so presented in Table 2.

In 1938 the crested wheatgrass plants were small but well established and their increase in basal cover by 1940 and 1948 was due mainly to increase in size of plants rather than to the establishment of new seedlings. As indicated by the data in Table 2, crested wheatgrass dominated all sections of the transect by 1948. This dominance was very largely at the expense of western couchgrass (*Agropyron Smithii* Rydb.). The latter had originally dominated the area but by 1948 it was reduced to a few scattered plants in the moister locations and had disappeared entirely from the drier portions of the site. Wild barley (*Hordeum jubatum* L.), sweet grass (*Hierochloe odorata* (L.) Wahl.), and goldenrod (*Solidago* spp.) were initially present near the edge of the slough but were crowded out by 1948. Smartweed (*Persicaria* sp.) was the principal broad-leaved plant in the moister sections of the transect and appeared to be little affected by competition

TABLE 2.—BASAL COVER IN PER CENT OF ALL SPECIES PRESENT IN THE BELT TRANSECT IN 1938, 1940, AND 1948, AND THE RATIO OF THE BASAL COVER OF CRESTED WHEATGRASS TO ALL OTHER SPECIES AT EACH DATE OF CHARTING

Transect sections	Year	Basal cover in per cent									Ratio crested wheatgrass to all others
		Crested wheat-grass	Western couch-grass	Wild barley	Sweet grass	Beaked sedge	Golden-rod	Smart-weed	Yarrow	Total	
A, B, and C	1938	1.67	14.67	—	—	—	—	—	—	16.34	1 : 8.78
	1940	5.07	27.18	—	—	—	—	—	—	32.25	1 : 5.36
	1948	18.19	2.05	—	—	—	0.10	—	0.07	20.41	1 : 0.12
D, E, and F	1938	0.67	13.37	—	—	0.02	—	0.22	—	14.28	1 : 20.31
	1940	4.22	29.11	—	—	—	—	0.60	—	33.93	1 : 7.04
	1948	16.80	0.54	—	—	0.34	1.03	0.30	—	19.01	1 : 0.13
G, H, and I	1938	3.07	1.20	1.00	0.30	—	0.07	1.71	—	7.35	1 : 1.39
	1940	13.73	2.62	0.03	0.78	—	0.09	1.06	—	18.31	1 : 0.33
	1948	24.31	—	—	—	0.30	—	1.29	—	25.90	1 : 0.07

from the crested wheatgrass. It is of interest that beaked sedge (*Carex rostrata* Stokes) was able to invade the lower sections and goldenrod and yarrow (*Achillae lanulosa* Nutt.) the dry upper sections of the transect.

Quadrats 1 and 2.—Quadrat No. 1 was located in the centre of the shallow slough and No. 2 at the edge. Both of these quadrats were dominated by wild barley in 1938. Table 3 records the changes in basal cover as measured from 1938 to 1948, and Figure 1 illustrates the changes that occurred in quadrat 2.

Wild barley is a common species on low-lying soils that often show a high content of soluble salts. Observations indicate that it is resistant to flooding. Bolton and McKenzie (3) have reported that crested wheatgrass is readily killed out by short periods of spring flooding. No accurate records of surface conditions were taken at Mortlach from 1940 to 1948 but flooding likely occurred in the slough—probably in 1942. This would account for the disappearance of crested wheatgrass in quadrat No. 1. However, the reason is not apparent why wild barley did not repossess the quadrat after the crested wheatgrass died out since its method of seed dispersal permits rapid invasion of denuded area. Probably invasion by wild barley did occur and it was followed in the succession by other moisture-loving species, namely, beaked sedge, fowl meadow grass (*Poa pallustris* L.), Baltic rush (*Juncus ater* Rydb.), and smartweed. In any case the data presented in Table 3 show that in the absence of flooding crested wheatgrass eliminated wild barley almost entirely in two years in both quadrats No. 1 and No. 2.

Quadrats 3, 4, 5, and 6.—These quadrats were located on the west-facing slope up from the shallow slough. The soil was high in soluble salts showing 0.155 per cent in the 0-6 inch layer and 1.768 per cent in the 6-12 inch layer (see Table 1). Alkali grass was the dominant species in quadrats 4, 5, and 6 and alkali cordgrass in quadrat 3. Both of these species are regarded as being alkali-tolerant and are indigenous to soils high in soluble salt content. The data recorded in basal cover for the above quadrats are summarized in Table 4.

The relationship between crested wheatgrass and alkali grass as summarized in Table 4 is of particular interest. Alkali grass dominated quadrats 4 and 6 in 1938 but was of relatively minor importance in quadrats 3

TABLE 3.—BASAL COVER IN PER CENT OF ALL SPECIES PRESENT IN QUADRAT NUMBERS 1 AND 2 AND RATIO OF CRESTED WHEATGRASS TO ALL OTHER SPECIES

Quadrat No.	Year	Basal cover in per cent							Ratio crested wheatgrass to all others
		Crested wheatgrass	Wild barley	Beaked sedge	Fowl meadow grass	Baltic rush	Smart-weed	Total	
1	1938	1.67	10.70	0.07	—	—	—	12.44	1 :6.45
	1940	10.90	1.70	0.03	—	—	—	12.63	1 :0.16
	1948	0.00	0.00	2.20	4.39	0.76	3.04	10.39	—
2	1938	5.22	8.72	—	—	—	—	13.94	1 :1.67
	1940	16.56	0.16	—	—	—	—	16.72	1 :0.01
	1948	29.28	—	—	—	—	—	29.28	—

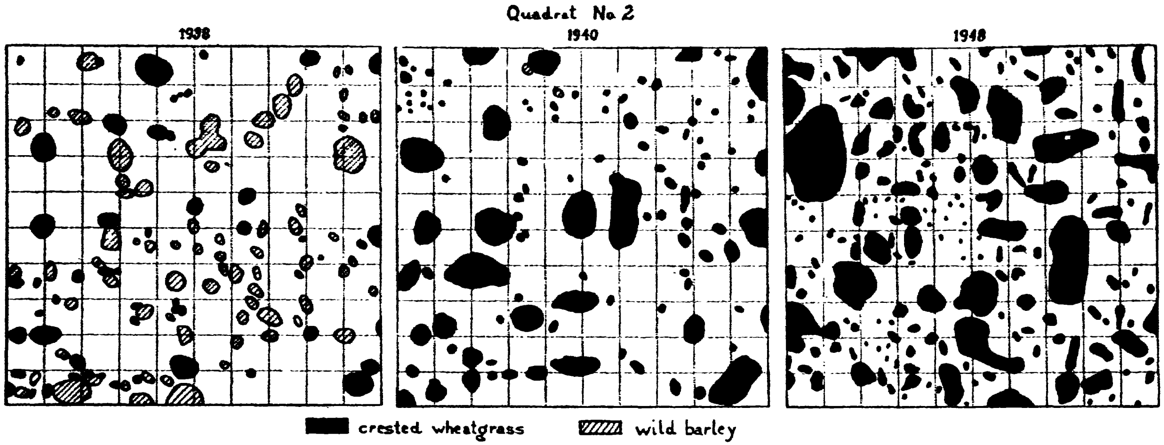

FIGURE 1. Charts of basal cover on quadrat 2 as recorded in 1938, 1940, and 1948.

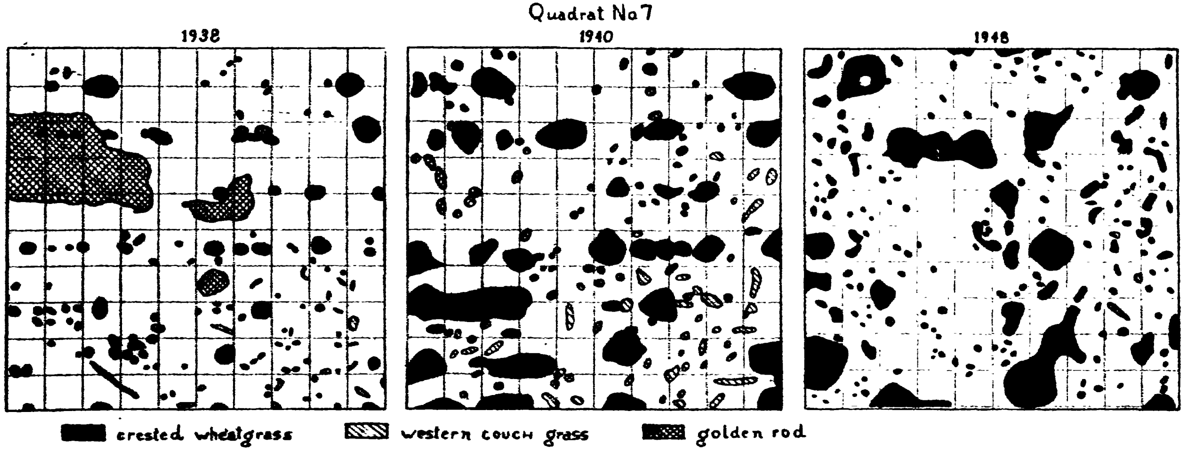

FIGURE 3. Charts of basal cover on quadrat 7 in 1938, 1940, and 1948.

TABLE 4.—BASAL COVER IN PER CENT OF ALL SPECIES PRESENT IN QUADRATS 3, 4, 5, AND 6 AS RECORDED IN 1938, 1940, AND 1948

Quadrat	Year	Basal cover in per cent						Ratio crested wheatgrass to all others
		Crested wheatgrass	Alkali grass	Alkali cordgrass	Wild barley	Pasture sage	Total	
3	1938	2.70	2.87	9.14	—	0.04	14.75	1 : 4.46
	1940	10.85	7.10	7.16	—	0.04	25.15	1 : 1.32
	1948	9.96	15.81	—	—	0.41	26.18	1 : 1.63
4	1938	3.90	24.80	0.44	—	—	29.14	1 : 6.47
	1940	14.22	27.18	—	—	—	41.40	1 : 1.90
	1948	14.83	20.47	—	—	—	35.30	1 : 1.38
5	1938	3.54	1.44	—	1.00	—	5.98	1 : 0.69
	1940	11.60	3.52	—	—	—	15.12	1 : 0.30
	1948	11.08	26.91	—	—	—	37.99	1 : 2.43
6	1938	2.90	14.17	—	0.19	—	17.26	1 : 4.95
	1940	9.47	23.16	—	—	—	32.63	1 : 2.45
	1948	13.23	16.81	—	—	—	30.04	1 : 1.27

and 5. By 1948 it had not only maintained its importance in quadrats 4 and 6 but had become the principal species in 3 and 5. In all cases crested wheatgrass increased rapidly in basal cover from 1938 to 1940 and remained relatively stable from 1940 to 1948. A careful examination of Figure 2 shows that, although certain of the original crested wheatgrass plants had disappeared by 1948, new ones had developed from shattered seed. The general conclusion reached is that crested wheatgrass will not crowd out alkali grass but it can become established and will make satisfactory growth in vigorous stands of alkali grass.

Alkali cordgrass had completely disappeared by 1948, as had also the few original plants of wild barley. These effects may have been caused by increased pressure from either alkali grass or crested wheatgrass. Probably the crested wheatgrass replaced the alkali cordgrass since the latter originally was growing in association with the alkali grass. Adequate check plots containing no crested wheatgrass would have been very useful in this instance. Pasture sage increased in quadrat 3 although it did not invade quadrats 4, 5, or 6.

Quadrats 7, 8, 9, and 10.—These quadrats were located on dry upland soil with a low content of soluble salts (see Table 1). They were charted to study competition between crested wheatgrass and goldenrod on quadrats 7, 8, and 9, and pasture sage on quadrat 10. Both species are common perennial pasture weeds. Unfortunately, quadrat 10 could not be located in 1948 and hence only data for 1938 and 1940 were recorded. Table 5 presents a summary of data on per cent basal cover and Figure 3 illustrates the chart taken from quadrat 7.

As indicated in Table 5, crested wheatgrass increased from 1938 to 1940 and then remained relatively constant in basal cover. Goldenrod decreased in all quadrats during the 10-year period and the decrease was particularly marked in quadrat 7 where the stand of this weed was very

TABLE 5.—BASAL COVER IN PER CENT OF ALL SPECIES PRESENT IN QUADRATS 7, 8, 9, AND 10 IN THE YEARS 1938, 1940, AND 1948

Quadrat No.	Year	Basal cover in per cent							Ratio crested wheatgrass to all others
		Crested wheat-grass	Western couch-grass	Sweet grass	Wild barley	Golden-rod	Pasture sage	Total	
7	1938	5.18	1.30	—	0.08	9.32	—	15.88	1 : 2.07
	1940	16.34	2.10	—	—	1.50	—	19.94	1 : 0.22
	1948	16.22	0.74	—	—	0.50	—	17.46	1 : 0.08
8	1938	3.76	—	1.86	0.04	1.04	—	6.70	1 : 0.78
	1940	13.08	—	—	—	1.54	—	14.66	1 : 0.12
	1948	16.22	—	—	—	0.49	—	17.12	1 : 0.06
9	1938	5.99	—	—	—	1.10	0.08	7.17	1 : 0.20
	1940	18.98	—	—	—	0.10	0.15	19.23	1 : 0.01
	1948	18.99	—	—	—	0.04	0.92	19.95	1 : 0.05
10	1938	3.76	0.08	—	—	—	8.48	12.32	1 : 2.28
	1940	14.90	0.04	—	—	—	0.48	15.42	1 : 0.03

heavy in 1938. In no case, however, was goldenrod completely eliminated. The two species of goldenrod present (*Solidago dumetorum* Lunell) and (*S. Lunellii* Rydb.) are vigorous, rather tall-growing species characteristic of abandoned farm-lands and favoured locations in native sod. Invasion by goldenrod was noted previously in the discussion of the transect (see Table 2). Unfortunately, the species was not determined in that case. It is possible that it was *S. missouriensis* Nutt. which is a common species on dry upland soils and may have better competitive qualities in relation to crested wheatgrass than the species found in quadrats 7, 8, and 9.

Traces of wild barley in quadrats 7 and 8 were quickly replaced. Sweet grass, as found in quadrat 8, also had disappeared by 1940. The situation with regard to pasture sage is not clear. In quadrat 3 (see Table 4) and in quadrat 9 it increased in basal cover. However, in these cases the increase was due to increase in size of small plants established previous to the first charting in 1938. On the other hand, in quadrat 10, there was a marked reduction in the basal cover of pasture sage from 1938 to 1940 and this agrees with general observations from field seedings as described in the review of literature. It is regrettable that it was not possible to re-locate quadrat 10 in 1948 and that additional quadrats containing pasture sage were not established in 1938.

Webb Area

The quadrats at Webb have been separated into two groups for the purposes of discussion and presentation of data. Quadrats 11 to 15, inclusive, were located and charted to study the effect of crested wheatgrass on established stands of hoary sagebush (*Artemisia cana* Pursh). The second group, quadrats 16 to 24, inclusive, were intended to supplement the Mortlach data by including additional common native species and duplicating others.

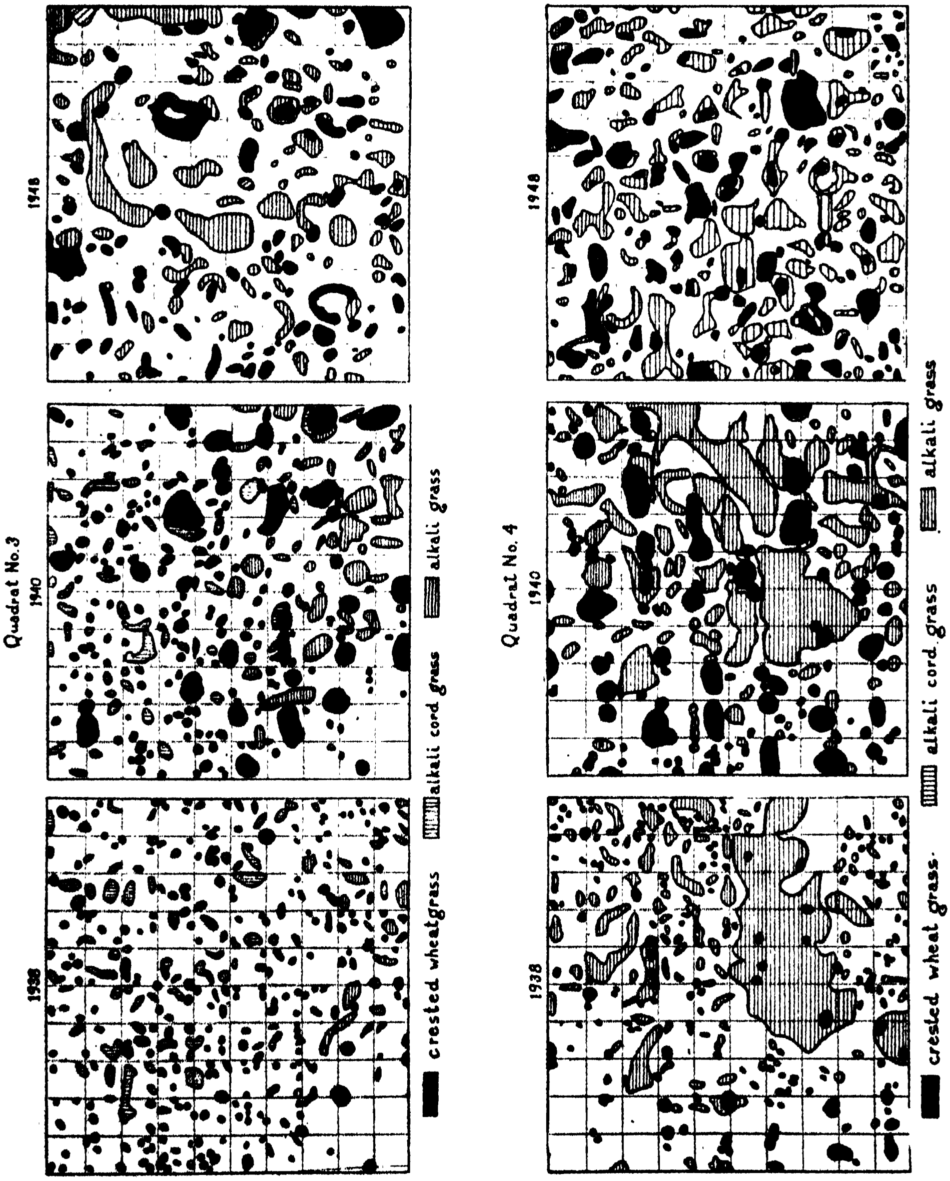

FIGURE 2. Charts of basal cover on quadrats 3 and 4 as recorded in 1938, 1940, and 1948.

Quadrats 11 to 15, inclusive.—Basal area data are presented in Table 6, and Figure 4 represents chartings made on quadrat 14 which is fairly typical of the group.

The data presented in Table 6 show that crested wheatgrass can become established in a stand of hoary sagebush plants and that it will attain a normal stand in 8 years or less. Competition between the two species does not appear to be severe, possibly because of different root systems. However, any competitive advantage apparently is in favour of crested wheatgrass. Very few new plants of hoary sagebush became established and in quadrats where the number of sagebush plants was high in 1940 there had been a considerable decrease by 1948 (see Figure 4). On the other hand, many new seedlings of crested wheatgrass invaded the quadrats especially where the original number was very low.

TABLE 6.—BASAL COVER IN PER CENT OF ALL SPECIES PRESENT IN QUADRATS 11, 12, 13, 14, AND 15 IN THE YEARS 1940 AND 1948

Quadrat	Year	Basal cover in per cent					Ratio crested wheatgrass to all others
		Crested wheat-grass	Hoary sage-bush	Pasture sage	Broom-weed	Total	
11	1940	1.12	0.64	—	—	1.76	1 : 0.57
	1948	14.16	3.56	0.08	—	17.80	1 : 0.26
12	1940	0.41	1.22	0.02	—	1.65	1 : 3.31
	1948	16.76	3.60	0.40	—	20.76	1 : 0.24
13	1940	0.14	1.84	0.23	—	2.21	1 : 14.79
	1948	12.84	2.72	0.52	1.60	17.68	1 : 0.38
14	1940	0.09	1.70	—	—	1.79	1 : 18.89
	1948	11.64	4.16	—	0.52	16.32	1 : 0.40
15	1940	2.10	0.39	—	—	2.49	1 : 0.19
	1948	14.72	3.44	—	—	18.16	1 : 0.23

There was some indication that pasture sage could invade the quadrats and also improve its position when originally present. However, it did not become an important constituent in any case. Broomweed (*Gutierrezia diversifolia* Greene) invaded both quadrats 13 and 14 and it is possible that this species may have a competitive advantage over crested wheatgrass.

Quadrats 16 to 24, inclusive.—Quadrats 16 to 24, inclusive were located on an extremely dry knoll which had never been cultivated. Table 7 presents data on basal cover and Figure 5 represents the chartings made on quadrat 16.

The data in Table 7 indicate two very strong competitors in relation to crested wheatgrass. Both blue grama grass and involute sedge (*Carex Eleocharis* Bailey) maintained or increased their basal cover from 1940 to 1948 in practically all quadrats where they occurred. Involute sedge in particular gained considerably. The above gains, however, were not made at the expense of crested wheatgrass. The latter increased very markedly in all quadrats except No. 19 where few seedlings of this species occurred

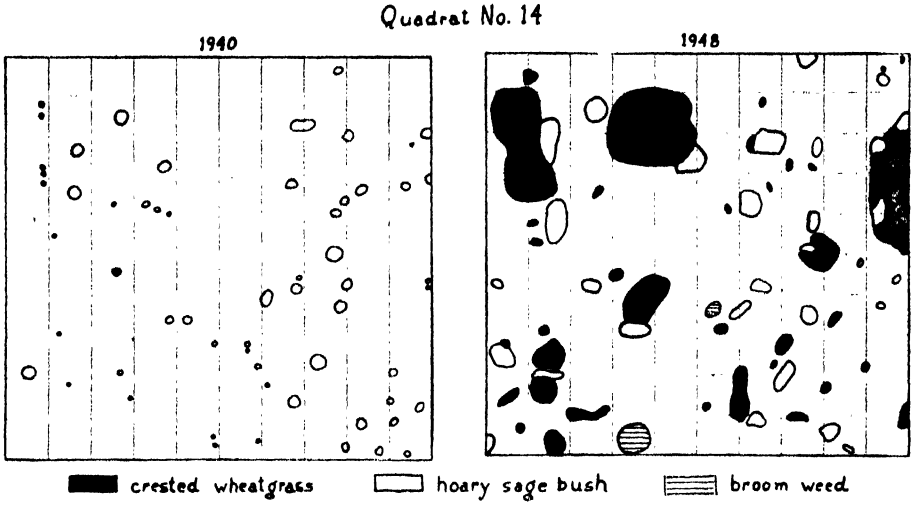

FIGURE 4. Charts of basal cover data on quadrat 14 in 1940 and 1948.

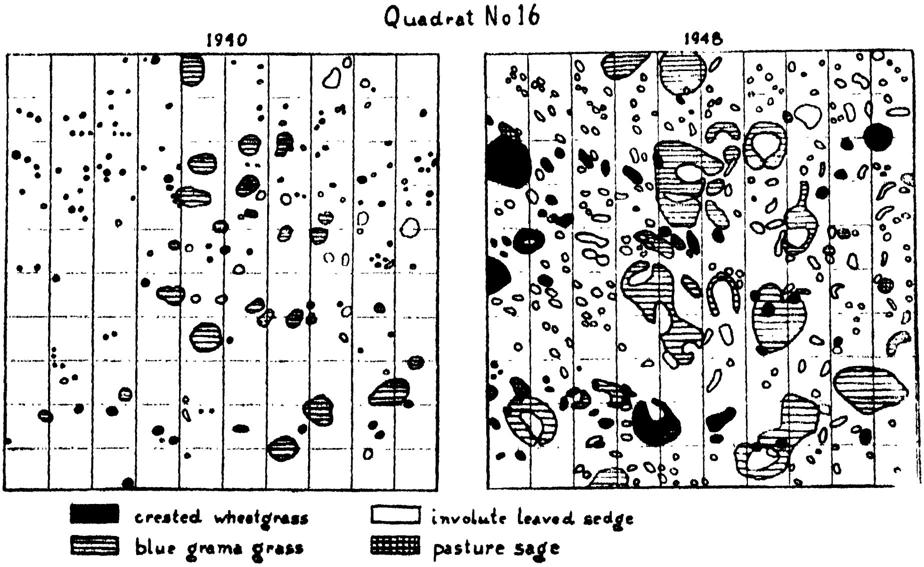

FIGURE 5. Charts of basal cover data on quadrat 16 in 1940 and 1948.

and a very thick stand of blue grama grass was present in 1940. As stated previously, this group of quadrats was in a very dry location, the basal cover in 1940 being very low and in a badly depleted condition. Hence, it is likely that much of the recorded increase was due to the occupation of bare ground and that little competition actually took place. If so, then it is probable that the cover attained by 1948 is about the maximum possible. A further charting at a later date should indicate whether crested wheatgrass will give way to blue grama grass and involute sedge, or *vice versa*, or whether all three species will retain their relative positions as of 1948. Common speargrass generally maintained its position in the quadrats but in no case was it abundant enough to be very important. Western couch-

TABLE 7.—BASAL COVER IN PER CENT FOR SPECIES PRESENT IN QUADRATS 16 TO 24, INCLUSIVE, IN THE YEARS 1940 AND 1948

Quadrat No.	Year	Basal cover in per cent										Ratio crested wheatgrass to all others
		Crested wheat-grass	Blue grama grass	Western couch-grass	Common spear-grass	Involute-leaved sedge	Thread-leaved sedge	Pasture sage	Nuttall's atriplex	Prairie sage	Total	
16	1940	0.56	2.35	0.07	—	1.54	—	—	—	—	4.52	1 : 7.07
	1948	4.08	11.11	—	—	5.28	—	0.64	—	—	21.11	1 : 4.17
17	1940	1.33	13.11	—	0.12	0.74	—	—	—	—	15.30	1 : 10.50
	1948	7.08	26.64	—	0.32	1.04	—	0.36	—	—	35.44	1 : 4.01
18	1940	1.51	18.61	0.02	—	0.17	—	—	—	—	20.31	1 : 12.45
	1948	6.94	19.64	—	—	0.76	—	—	—	—	27.34	1 : 2.94
19	1940	0.20	31.61	0.68	—	3.27	—	—	—	0.38	36.14	1 : 179.70
	1948	0.08	26.84	—	0.52	8.44	—	0.48	—	1.08	37.44	1 : 467.00
20	1940	1.20	—	0.23	1.12	—	—	—	—	—	2.55	1 : 1.12
	1948	5.68	—	1.64	0.80	—	—	3.60	—	—	11.72	1 : 1.06
21	1940	1.29	—	0.31	1.73	0.08	—	—	—	—	3.41	1 : 1.64
	1948	14.84	—	—	1.92	0.16	—	—	—	—	16.92	1 : 0.14
22	1940	0.68	—	3.72	0.06	0.17	—	—	—	0.27	4.90	1 : 6.21
	1948	8.36	—	0.16	0.48	2.56	0.32	3.20	—	2.08	17.16	1 : 1.05
23	1940	0.73	—	5.35	—	0.04	—	—	—	—	6.12	1 : 7.38
	1948	9.12	0.04	—	—	2.76	—	1.40	—	—	13.32	1 : 0.46
24	1940	1.18	—	0.03	0.12	0.09	—	—	4.81	—	6.23	1 : 4.28
	1948	12.16	—	—	—	0.76	—	0.52	2.56	—	16.00	1 : 0.32

grass reacted much the same as in the Mortlach area and practically disappeared by 1948, except on No. 20 where very few other native species were present. The one plant of Nuttall's atriplex (*Atriplex Nuttallii* S. Wats.) decreased in size to about one-half and no new plants became established. The increase of prairie sage (*Artemisia gnaphalodes* Nutt.) on quadrats 19 and 22 is of interest, as is the invasion of pasture sage in seven of the nine quadrats. This reaction may be associated with the same reasons suggested for the increase or maintenance of stands of blue grama grass and involute sedge. That is, that the maximum cover for the area has been attained only recently and hence that competition between species was not severe during the period studied.

DISCUSSION

In a general analysis of the results presented so far a summary of basal cover in per cent is helpful. The first and final chartings only have been considered in Table 8 and quadrats 1 and 10 have been omitted since the former undoubtedly was affected severely by flooding and no 1948 data were available for the latter.

An appraisal of the data presented in Table 8 shows that there was a large increase in the basal area occupied by crested wheatgrass from the beginning to the end of the period studied. This applies at both Mortlach and Webb, although the increase was much greater at Webb. Such an increase is to be expected only from a strong competitor. The crested wheatgrass plants were very small and relatively undeveloped when first charted and would have been eliminated or at least subdued if the native species had been more aggressive. Most of the increase was due to the growth of established plants but in some cases a significant increase from the growth of new seedlings was noted. In regard to native species, there was an average decrease of 19 per cent in basal cover at Mortlach and an average increase of 54 per cent at Webb. This is evidence, as suggested previously, that the Webb area was in a more depleted condition in 1940 than the Mortlach area was in 1938. The general effect on the average total basal cover was almost to double it at Mortlach and nearly triple it at Webb. This over-all increase in basal cover was entirely due to crested wheatgrass at Mortlach since there was an actual decrease of other species. At Webb crested wheatgrass also contributed largely to the general increase

TABLE 8.—AVERAGE BASAL COVER IN PER CENT AND PER CENT INCREASE IN BASAL COVER FOR 11 QUADRATS[1] AT MORTLACH AND 14 QUADRATS AT WEBB

Constituents of basal cover	Mortlach area			Webb area		
	1938	1948	Per cent increase	1940	1948	Per cent increase
Crested wheatgrass	3.51	17.19	390	0.90	9.89	1000
All other species	10.02	8.13	− 19	6.91	10.62	54
Total basal cover	13.53	25.32	87	7.81	20.51	163

[1] The 3 divisions of the transect as shown in Table 3 were considered 3 quadrats in calculating the data for this table.

since other species increased only 54 per cent while the total increase was 163 per cent. Apparently crested wheatgrass is a strong competitor that can at least maintain its position as compared to certain species and increase greatly at the expense of others.

SUMMARY

1. Crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.) of the Fairway variety was seeded into established stands of native perennial species at two locations in Southern Saskatchewan. Changes in basal cover were determined at the end of two years and ten years at one location and at the end of eight years at the other.

2. Crested wheatgrass made very large gains in basal cover at both locations. These gains were greatest, relative to the native species, where the original cover was the lowest.

3. Wild barley (*Hordeum jubatum* L.) and sweet grass (*Hierochloe odorata* (L.) Wahl.) were eliminated within two years. Alkali cordgrass (*Spartina gracilis* Trin.), western couchgrass (*Agropyron Smithii* Rydb.), and goldenrod (*Solidago* sp.) disappeared or were greatly reduced but over a longer period. Alkali grass (*Distichlis stricta* (Torr.) Rydb.), blue grama grass (*Bouteloua gracilis* (H.B.K.) Lag.), involute sedge (*Carex Eleocharis* Bailey), common speargrass (*Stipa comata* (Trin.) Rupr.), hoary sagebush (*Artemisia cana* Pursh), and smartweed (*Persicaria* sp.) were controlled in that they did not prevent crested wheatgrass from taking an important and sometimes dominant place in the habitat. Mature plants of pasture sage (*Artemisia frigida* Willd.) were greatly reduced in basal cover but there were minor invasions by seedlings of this species.

4. It was concluded that crested wheatgrass is a strong competitor with perennial native species under the conditions studied.

ACKNOWLEDGMENTS

The writers wish to express their appreciation to J. L. Doughty, Soil Research Laboratory, Swift Current, Saskatchewan, for the analysis of soil samples, and M. Kilcher, Experimental Station, Swift Current, Saskatchewan, for assistance in charting the quadrats in 1948. Helpful criticism of the manuscript was received from J. B. Campbell, Experimental Station, Swift Current, Saskatchewan, R. T. Coupland, Department of Plant Ecology, University of Saskatchewan, Saskatoon, Saskatchewan, and from W. J. White and R. P. Knowles, both of the Dominion Forage Crops Laboratory, Saskatoon, Saskatchewan.

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APPENDIX

Common and scientific names of species occurring in the transect and quadrats of the study areas:

Alkali cordgrass	*Spartina gracilis* Trin.
Alkali grass (Saltgrass)	*Distichlis stricta* (Torr.) Rydb.
Baltic rush	*Juncus ater* Rydb.
Beaked sedge (Retrorse sedge)	*Carex rostrata* Stokes
Blue grama grass	*Bouteloua gracilis* (H.B.K.) Lag.
Broomweed	*Gutierrezia diversifolia* Greene
Common speargrass	*Stipa comata* Trin. and Rupr.
Crested wheatgrass	*Agropyron cristatum* (L.) Gaertn.
Fowl meadow grass	*Poa palustris* L.
Goldenrod, low	*Solidago missouriensis* Nutt.
Goldenrod, Lunell's	*Solidago Lunellii* Rydb.
Goldenrod, prairie	*Solidago dumetorum* Lunell
Hoary sagebush	*Artemisia cana* Pursh
Involute-leaved sedge	*Carex Eleocharis* Bailey
Nuttall's atriplex	*Atriplex Nuttallii* S. Wats.
Pasture sage	*Artemisia frigida* Willd.
Prairie sage	*Artemisia gnaphalodes* Nutt.
Smartweed	*Persicaria* sp.
Sweet grass	*Hierochloe odorata* (L.) Wahl.
Thread-leaved sedge (Niggerwool)	*Carex filifolia* Nutt.
Western couchgrass (Western wheatgrass)	*Agropyron Smithii* Rydb.
Wild barley	*Hordeum jubatum* L.
White sage	*Eurotia lanata* (Pursh) Moq.
Yarrow	*Achillea lanulosa* Nutt.

SOME FURTHER OBSERVATIONS ON SMALL BITTER CHERRY[1]

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Further information obtained on small bitter cherry indicates that this disease is becoming increasingly important in the Okanagan Valley of British Columbia. Transmission has now been obtained. The disease has been found in the Lambert variety of sweet cherry in addition to the Bing variety in which it was previously reported (1). The number of natural infections has increased slowly but steadily.

TRANSMISSION

Transmission was obtained for the first time in a single tree in 1949, as a result of inoculation made in 1941. As previously reported eight trees were inoculated by grafting or budding with diseased material in 1941 and 1942, and two trees were inoculated by budding in 1944. The tree in which transmission was obtained in 1949 was one of those inoculated in 1941. Scions from a diseased Bing tree were successfully grafted on to two branches of a young Bing tree, the remainder of which was allowed to grow. One inserted scion grew into a small branch which has consistently produced only normal cherries. The other inserted scion grew into a branch which is now twenty feet high with strong lateral branches, and which was definitely affected with small bitter cherry in 1947, 1948, and 1949. In all three years this branch carried a good crop and the majority of the cherries were normal. In 1947, small bitter cherry was evident in only a few fruits on the lower part of this branch. In 1948 and 1949, the disease could be observed in a small number of cherries scattered through the branch. Transmission first became evident in 1949 when small bitter cherry was observed on the fruits on small branches growing below the points of inoculation. No signs of transmission were observed at this time on the remainder of the inoculated tree which produced a good crop of normal cherries. The other inoculated trees, some of which are still on hand, have shown no signs of transmission.

SYMPTOMS IN THE LAMBERT VARIETY

The symptoms of small bitter cherry in the Lambert variety (Figure 1) are essentially the same as the symptoms in the Bing variety which were described previously. Only three affected Lambert trees have been observed. Two of these were new infections in 1949 while the other has been infected for several years. In these trees some of the cherries were apparently entirely normal and even in the tree that has been infected for several years some branches produced only normal cherries. In some branches small and apparently normal cherries occurred together. In some smaller branches all of the fruits were abnormal. At normal picking time most of the small cherries were bright red like immature cherries,

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[1] Contribution No. 1040 from Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

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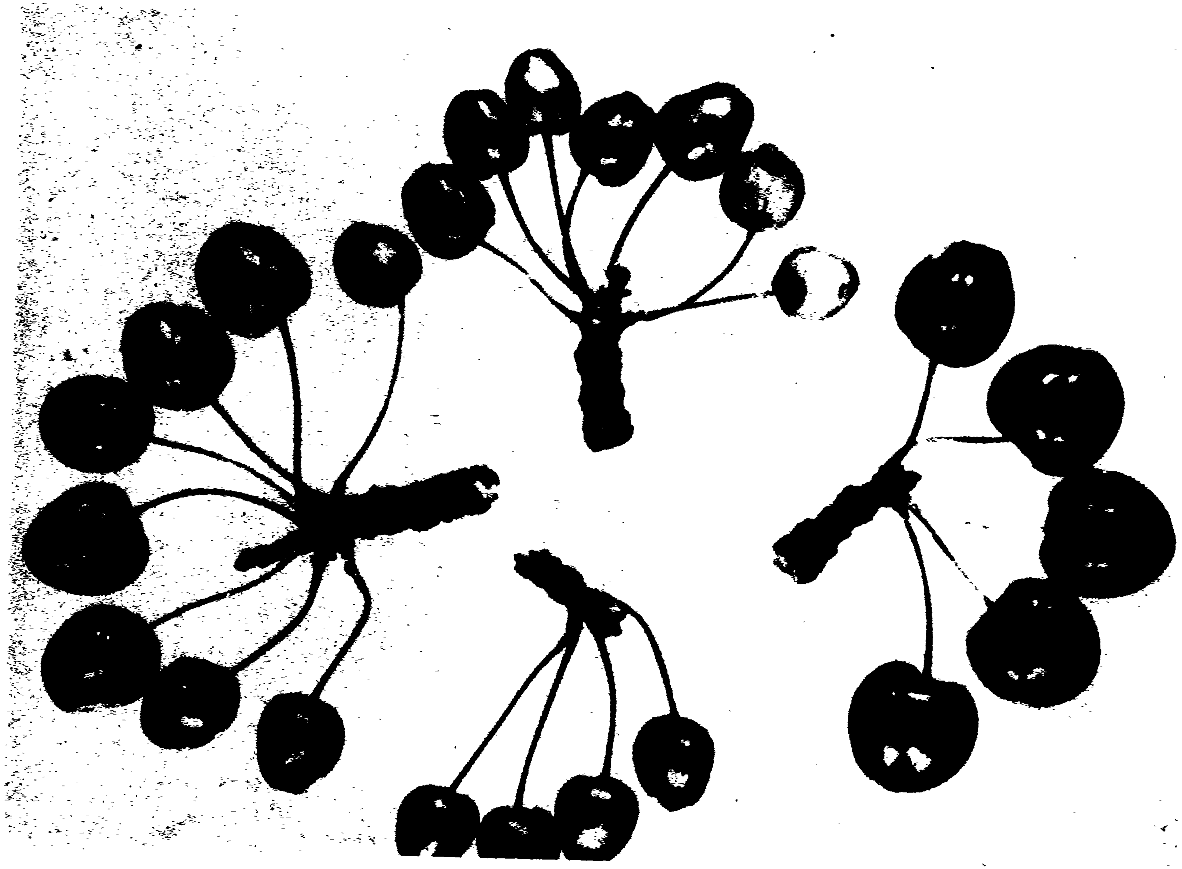

(Photograph by S. R. Cannings)

FIGURE 1. Small bitter cherry in the Lambert variety. All cherries from the same tree.

while a few were light pink and much under-developed. The small cherries were oval in side view, and rounded, not triangular, when viewed from the distal end. In taste the first impression was bitterness, soon superseded by a positively objectionable flavour. Most of the small cherries had the gloss of normal ones. It is particularly noteworthy that the Lambert tree that has been diseased for years produced many cherries that appeared entirely normal. No experimental work has been done on these Lambert trees but the symptoms in Lambert and in Bing are so similar that it appears almost certain that the same disease is involved in both varieties.

SPREAD WITHIN THE INDIVIDUAL TREE

The manner in which small bitter cherry spreads in or through individual trees is of both diagnostic and economic importance. The rate of spread within the individual tree has differed widely in different trees and sometimes in different years in a single tree. Observations made in four successive years have shown that the disease may remain in a naturally infected tree with negligible spread for as much as three years. On the other hand, very extensive spread was recorded one year in a tree in which negligible spread had been recorded the preceding year.

GENERAL

The number of new natural infections and their locations are cause for concern if not alarm. Up to 1946, eight infected trees had been observed in five orchards. By 1949, twenty-two infected trees had been recorded in fourteen orchards. These infections were scattered through the Oliver and Osoyoos districts in the southern part of the Okanagan Valley. No infections have been found farther north.

Small bitter cherry in sweet cherry may be caused by the same virus that causes Western X in peach, but the available information does not warrant any definite statement of relationship. Small bitter cherry has been found only in the part of the Okanagan Valley where Western X is fairly common in peach. Limited experimental work has not demonstrated any relationship. The exact relationship between small bitter cherry and other diseases producing abnormal fruits in sweet cherry is not yet known.

SUMMARY

1. Small bitter cherry is becoming increasingly important in the Okanagan Valley of British Columbia.

2. Transmission has been obtained. Transmission became evident eight years after inoculation, indicating an unusually long incubation period.

3. The disease has been found in the Lambert variety in addition to the Bing variety in which it was previously reported.

4. The symptoms in the Lambert variety are essentially the same as those in the Bing variety.

5. The rate of spread of the disease in the individual tree has differed widely in different trees and sometimes in different years in a single tree.

6. The number of naturally-occurring infections is increasing steadily.

7. Small bitter cherry has only been found where Western X is fairly common in peach but limited experimental work has not demonstrated any relationship.

8. The relationship between small bitter cherry and other diseases causing abnormal fruits in sweet cherry is not yet known.

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SOIL FERTILITY STUDIES

II. MANURE AND FERTILIZERS FOR POTATOES AT CHARLOTTETOWN, P.E.I.[1]

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[Received for publication July 27, 1950]

OUTLINE OF EXPERIMENT

An investigation to obtain information with respect to the use of manure and chemical fertilizers for the production of potatoes in Prince Edward Island was begun at the Dominion Experimental Station at Charlottetown in 1927. The soil on which the plots were located was a brownish-red fine sandy loam. Analysis of 24 samples of surface soil collected at the beginning of the experiment showed an average pH value of 5.0 and an average nitrogen content of 0.17 per cent.

The plot area was laid out as shown in Figure 1. There were 24 plots, each 13.2 feet by 82.5 feet (1/40th acre), with 4-foot paths between plots. On plots 1 to 6, potatoes were grown continuously. On the remaining 18 plots, a three-year rotation of potatoes, grain and clover was followed. The rotation was begun in 1927 with potatoes on plots 7 to 12 and wheat on plots 13 to 24. In 1928, wheat was grown on plots 13 to 18 and potatoes on plots 19 to 24. Wheat was grown up to and including 1936. Barley was sown in 1937 and has been grown as the grain crop since that time.

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[1] Joint contribution from the Division of Chemistry, Science Service and the Division of Field Husbandry, Experimental Farms Service. Scientific contribution No. 183, Division of Chemistry.

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Figure 1.—Plan of Experiment

(North)

							L	I	M	E		S	T	R	I	P							
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
	D	O	U	B	L	E		T	R	E	A	T	M	E	N	T		S	T	R	I	P	
Continuous Potatoes						Rotation Potatoes—'48						Rotation Barley—'48						Rotation Clover—'48					

Plots 1, 7, 13, 19—Check
" 2, 8, 14, 20—Manure
" 3, 9, 15, 21—NPK (NaNO$_3$)
" 4, 10, 16, 22—NPK (NH$_4$)$_2$SO$_4$)
" 5, 11, 17, 23—Manure + NPK (NaNO$_3$)
" 6, 12, 18, 24—Manure + NPK ((NH$_4$)$_2$SO$_4$)

TABLE 1.—COMPARISON OF YIELDS OF POTATOES GROWN CONTINUOUSLY WITH YIELDS OF POTATOES GROWN IN ROTATION UNDER DIFFERENT FERTILIZER TREATMENTS

(Average for 22 years—1927–48 inclusive)

Treatment	Continuously		In rotation	
	Yield (bu./ac.)	% market.	Yield (bu./ac.)	% market.
1. Check	77	60	143	68
2. Manure	249	84	270	84
3. NPK ($NaNO_3$)	179	76	233	83
4. NPK ($(NH_4)_2SO_4$)	111	72	204	80
5. ½ Manure ½ NPK ($NaNO_3$)	239	82	262	84
6. ½ Manure ½ NPK ($(NH_4)_2SO_4$)	235	81	252	84

The following treatments have been used: (1) Check, no treatment; (2) 15 tons of manure per acre; (3) 1000 lb. of a 4–8–8 fertilizer per acre, using nitrate of soda as a source of nitrogen; (4) 1000 lb. of a 4–8–8 fertilizer per acre, using sulphate of ammonia as a source of nitrogen; (5) 7½ tons of manure and 500 lb. of 4–8–8 fertilizer per acre, using nitrate of soda as a source of nitrogen; (6) 7½ tons of manure and 500 lb. of a 4–8–8 fertilizer per acre, using sulphate of ammonia as a source of nitrogen. The treatments were not randomized but were applied to the plots in the order given above, plots 1, 7, 13 and 19 being the check plots. Beginning in 1941, an application of ground limestone at the rate of one ton per acre has been made to a 20-foot strip along the north end of the plots once every three years before the potato crop in every case. Beginning in 1945, the manure and fertilizers were applied at double the usual rate to a 20-foot strip along the south end of the plots.

With the design of the experiment as outlined above, it was possible to make the following comparisons: (1) the effect of growing potatoes continuously with growing them in rotation; (2) the effect of manure alone with fertilizers alone and with manure and fertilizers in combination; (3) the effect of $NaNO_3$ with $(NH_4)_2SO_4$ as a source of nitrogen; (4) the effect of application of ground limestone with no limestone; (5) the effect of different rates of application of the manure and fertilizers.

YIELDS

Average yields of potatoes for the period 1927 to 1948 are presented in Table 1 to compare the effect of potatoes grown continuously with that of potatoes grown in rotation under the different manure and fertilizer treatments. From these data, the following observations can be made: *(a)* The yields with each treatment were always higher where the potatoes were grown in rotation. *(b)* There was a larger percentage of marketable potatoes when grown in rotation on the check and NPK plots; where manure was used, this difference was slight. *(c)* The largest yields were obtained on the manured plots. *(d)* The yields were larger where manure alone was used than where half manure and half fertilizer was employed. *(e)* Larger yields were obtained where $NaNO_3$ was used than where $(NH_4)_2SO_4$ was the source of nitrogen; this difference was greater where these materials were used without manure.

TABLE 2.—COMPARISON OF YIELDS OF POTATOES GROWN CONTINUOUSLY WITH YIELDS OF POTATOES GROWN IN ROTATION UNDER DIFFERENT FERTILIZER TREATMENTS AND ON LIMED AND UNLIMED AREAS

(Average for 8 years—1941–48 inclusive—in bu./ac.)

Treatment	Continuously		In rotation	
	Unlimed	Limed	Unlimed	Limed
1. Check	49	76	111	138
2. Manure	239	262	268	292
3. NPK ($NaNO_3$)	135	162	224	247
4. NPK ($(NH_4)_2SO_4$)	41	133	168	235
5. ½ Manure ½ NPK ($NaNO_3$)	230	236	250	265
6. ½ Manure ½ NPK ($(NH_4)_2SO_4$)	232	256	244	273

TABLE 3.—COMPARISON OF YIELDS OF POTATOES GROWN CONTINUOUSLY WITH YIELDS OF POTATOES GROWN IN ROTATION UNDER DIFFERENT FERTILIZER TREATMENTS AND ON AREAS RECEIVING SINGLE AND DOUBLE APPLICATIONS OF THE TREATMENTS

(Average for 4 years—1945–48 inclusive—in bu./ac.)

Treatment	Continuously		In rotation	
	Single	Double	Single	Double
1. Check	59	75	128	160
2. Manure	275	357	329	392
3. NPK ($NaNO_3$)	121	225	263	343
4. NPK ($(NH_4)_2SO_4$)	29	41	194	223
5. ½ Manure ½ NPK ($NaNO_3$)	256	368	283	379
6. ½ Manure ½ NPK ($(NH_4)_2SO_4$)	252	357	289	370

Average results for the period 1941 to 1948 are presented in Table 2 to show the comparison of yields of potatoes grown continuously with those of potatoes grown in rotation under the different manure and fertilizer treatments and on limed and unlimed areas. These cover the period during which lime was applied to a part of the experimental area. The data show that, for this 8-year period, as for the 22-year period presented in Table 1, yields were higher where the potatoes were grown in rotation and the largest yields were obtained on the manured plots. In each case, yields were higher on the limed plots. The greatest increase due to the lime was on the plot receiving sulphate of ammonia without manure on the continuous potatoes block. Here the average yield of the unlimed plot was less than that of the unlimed check plot.

Average results for the period 1945–1948 are presented in Table 3 to show the comparison of yields of potatoes grown continuously with those of potatoes grown in rotation under the different manure and fertilizer treatments and on areas receiving single and double applications of the treatments. As in the previous comparisons the yields were larger where potatoes were grown in rotation and the largest yields were obtained where manure was applied. Where potatoes were grown continuously, the yields

TABLE 4.—AVERAGE YIELDS OF BARLEY UNDER VARIOUS TREATMENTS AND FOR DIFFERENT PERIODS

(Bushels per acre)

Treatment	1937–48	1942–48		1946–48	
	Unlimed single	Unlimed single	Limed single	Unlimed single	Unlimed double
1. Check	14	11	17	6	4
2. Manure	26	20	27	14	15
3. NPK ($NaNO_3$)	17	14	21	9	9
4. NPK ($(NH_4)_2SO_4$)	10	4	12	3	1
5. ½ Manure ½ NPK ($NaNO_3$)	21	17	25	15	13
6. ½ Manure ½ NPK ($(NH_4)_2SO_4$)	21	18	26	14	13

TABLE 5.—AVERAGE YIELDS OF CLOVER UNDER VARIOUS TREATMENTS AND FOR DIFFERENT PERIODS

(Tons per acre)

Treatment	1937–48	1942–48		1947–48	
	Unlimed single	Unlimed single	Limed single	Unlimed single	Unlimed Double
1. Check	0.72	0.64	1.25	0.80	0.67
2. Manure	1.70	1.48	2.68	1.52	2.15
3. NPK ($NaNO_3$)	0.74	0.64	1.85	0.91	0.85
4. NPK ($(NH_4)_2SO_4$)	0.46	0.33	1.82	0.00	0.00
5. ½ Manure ½ NPK ($NaNO_3$)	1.39	1.17	1.76	1.19	1.87
6. ½ Manure ½ NPK ($(NH_4)_2SO_4$)	1.29	1.05	2.17	1.11	1.72

from the plots receiving NPK with the nitrogen supplied by sulphate of ammonia were lower than on the untreated plots. In every case, yields were increased by the double treatment.

The average yields of barley under the different manure and fertilizer treatments comparing limed and unlimed areas and comparing areas receiving single with those receiving double applications of the treatments are presented in Table 4. In every case, the manure treatments gave the highest yield. The lowest yields were obtained where sulphate of ammonia was used without manure. In comparing limed with unlimed areas, the yields were higher on the limed plots. In comparing the effects of single and double treatments, average yields were usually slightly lower on the double treatments. Here it might be noted that the treatments were applied to the potato crop the year previous to the barley crop and resulted in larger yields of potatoes.

The average yields of clover under the different manure and fertilizer treatments, comparing limed and unlimed areas and comparing areas receiving single and those receiving double applications of the treatments are presented in Table 5. In all but one case (treatment 5, limed) yields were highest where manure was applied. Except where lime was used, yields were lowest on treatment number 4 where sulphate of ammonia was used without manure. In 1947 and 1948, no yields were reported on this treatment. Yields were considerably higher on the limed than on the unlimed treatments, the greatest increase occurring on treatment number 4. When the yields for the double treatment are compared with those from the single treatment, it is seen that the double treatment increased yields wherever manure was applied but not where manure was omitted.

SOIL COMPOSITION

Soil samples were collected from each section of all plots in the fall of 1948 and examined in the laboratory. In Table 6, the average results for reaction (pH), loss on ignition and nitrogen for the plots receiving the different manure and fertilizer treatments are presented. Since lime was added to certain of these plots and might be expected to affect the pH values, it was thought to be of interest to show, as well, the average pH values for the unlimed plots only. The average pH values ranged from 4.7 on the plots receiving sulphate of ammonia without manure to 5.5 on the plots receiving manure alone. Considering the unlimed plots only, these values were 4.3 and 5.3 respectively. It is evident that manure has increased, and sulphate of ammonia has decreased, pH values. Organic matter as measured by loss on ignition and nitrogen was highest on those plots to which manure had been applied and lowest average values were found on the check plots.

In Table 7, the average results of the same determinations for all plots receiving lime since 1941 are compared with those for plots receiving the

TABLE 6.—AVERAGE RESULTS OF ANALYSIS OF SOIL SAMPLES FROM THE PLOTS RECEIVING DIFFERENT MANURE AND FERTILIZER TREATMENTS

(Reaction, Loss on Ignition, Nitrogen)

Treatment	pH		L. on I. %	N %
	All plots	Unlimed plots		
1. Check	5.2	4.9	3.94	0.15
2. Manure	5.5	5.3	4.82	0.18
3. NPK ($NaNO_3$)	5.0	4.7	4.19	0.16
4. NPK ($(NH_4)_2SO_4$)	4.7	4.3	4.22	0.16
5. ½ Manure ½ NPK ($NaNO_3$)	5.3	5.0	4.81	0.18
6. ½ Manure ½ NPK ($(NH_4)_2SO_4$)	5.3	5.0	4.67	0.18

TABLE 7.—AVERAGE RESULTS OF ANALYSIS OF SOIL SAMPLES FROM LIMED, UNLIMED AND DOUBLE TREATMENT PLOTS

(Reaction, Loss on Ignition, Nitrogen)

Description	pH	L. on I. %	N %
Limed	5.7	4.45	0.17
Unlimed	4.9	4.39	0.16
Double treatment	4.8	4.49	0.17

TABLE 8.—AVERAGE RESULTS OF ANALYSIS OF SOIL SAMPLES FROM THE BLOCK IN POTATOES CONTINUOUSLY AND THE THREE BLOCKS IN CROP ROTATION

(Reaction, Loss on Ignition, Nitrogen)

Description	pH		L. on I. %	N %
	All plots	Unlimed plots		
Continuous	5.3	5.0	4.09	0.15
Rotation				
Potatoes, 1948	5.0	4.9	4.33	0.16
Grain, 1948	5.1	4.8	4.64	0.17
Hay, 1948	5.2	4.8	4.71	0.18

TABLE 9.—AVERAGE RESULTS OF ANALYSIS OF SOIL SAMPLES FROM THE PLOTS RECEIVING DIFFERENT MANURE AND FERTILIZER TREATMENTS

(Soluble phosphorus and exchangeable bases)

Treatment	Sol. P		Ex. CaO		Ex. MgO %	Ex. K_2O %
	All plots p.p.m.P	Unlimed plots p.p.m.P	All plots %	Unlimed plots %		
1. Check	15	13	0.103	0.061	0.004	0.007
2. Manure	44	37	0.145	0.104	0.009	0.010
3. NPK ($NaNO_3$)	37	32	0.109	0.067	0.003	0.008
4. NPK ($(NH_4)_2SO_4$)	37	36	0.091	0.047	0.002	0.008
5. ½ Manure ½ NPK ($NaNO_3$)	51	44	0.147	0.107	0.007	0.010
6. ½ Manure ½ NPK ($(NH_4)_2SO_4$)	51	47	0.138	0.089	0.006	0.009

normal treatment from the beginning of the experiment in 1927 and with those for plots receiving the double manure and fertilizer treatments since 1945. The samples from the plots which have received lime showed increased pH values. There appeared to be no significant differences in the results for loss on ignition and nitrogen due to these treatments.

In Table 8, the average results of the same determinations for all the plots on which potatoes have been grown continuously are presented in comparison with similar results for each block of plots on which the three-year rotation has been followed. The average pH values on the three blocks in the rotation were lower than that for the block continuously in potatoes but when the results for the unlimed plots alone are considered, the differences were small and probably not significant. The figures for loss on ignition and nitrogen indicate that the organic matter levels were slightly lower on the plots which were cultivated every year for potatoes.

In Table 9, the average results for readily soluble phosphorus and the exchangeable bases for the plots receiving the different manure and fertilizer treatments are presented. Since lime where applied affected the soluble phosphorus and exchangeable calcium contents (Table 10), average values

TABLE 10.—AVERAGE RESULTS OF ANALYSIS OF SOIL SAMPLES FROM LIMED AND UNLIMED PLOTS

(Soluble phosphorus and exchangeable bases)

Description	Sol. P. p.p.m. P	Ex. CaO %	Ex. MgO %	Ex. K_2O %
Limed	44	0.165	0.006	0.009
Unlimed	34	0.079	0.005	0.008

TABLE 11.—AVERAGE RESULTS OF ANALYSIS OF SOIL SAMPLES FROM THE BLOCK IN POTATOES CONTINUOUSLY AND THE THREE BLOCKS IN CROP ROTATION

(Soluble phosphorus and exchangeable bases)

Description	Sol. P		Ex. CaO		Ex. MgO %	Ex. K_2O %
	All plots p.p.m.P	Unlimed plots p.p.m.P	All plots %	Unlimed plots %		
Continuous	79	66	0.132	0.097	0.007	0.015
Rotation						
Potatoes, 1948	27	26	0.084	0.068	0.006	0.007
Grain, 1948	25	23	0.120	0.070	0.003	0.007
Hay, 1948	27	24	0.152	0.082	0.005	0.005

for these determinations on unlimed plots only are also presented. The phosphorus determined was that soluble in ammonium sulphate solution at pH 3·0 (modified Truog method) and it is apparent that the amounts present have been influenced by the treatments. Considering average values for all (limed and unlimed) plots, the lowest results (15 p.p.m. P) were found on the untreated plots. Applications of manure alone increased the readily soluble phosphorus considerably (to 44 p.p.m. P) as did those of inorganic phosphorus (treatments 3 and 4) though to a slightly less extent (37 p.p.m. P). Highest values for soluble phosphorus (51 p.p.m. P) were obtained on those plots where part of the added phosphorus came from manure and part from an inorganic source. The same picture is shown when only the unlimed plots are considered.

The effect of manure on the exchangeable base content of the soil samples was also quite pronounced. The figures in Table 9 show that highest average values for calcium (all plots), magnesium and potassium were found where manure was applied. The effect of the manure applications on potassium was not great, however. There is frequently a close relationship between the pH values of soils and their exchangeable lime content and this observation was true for the samples under consideration. Reference to Table 6 shows that the highest pH values were obtained on the plots receiving manure. The same relationship was found when the results for the unlimed plots were considered separately. The pH values and exchangeable calcium contents of the samples were increased where manure was applied. The effect of sulphate of ammonia in reducing the exchangeable calcium on unlimed plots was quite pronounced.

In Table 10 the average results of the same determinations for all plots receiving lime since 1941 are compared with those for plots receiving the normal treatment from the beginning of the experiment in 1927. (These determinations were not made on the samples from the plots receiving the double manure and fertilizer treatments since 1945). It is apparent that liming has increased the readily soluble phosphorus content of these samples. It is usually assumed that the liming of acid soils reduces the ability of iron and aluminum to fix phosphorus in a relatively insoluble form and this assumption has been supported by these results. As was to be expected, the applications of limestone greatly increased the exchangeable calcium content but did not affect the exchangeable magnesium and potassium.

In Table 11, the average results of the same determinations for all the plots on which potatoes have been grown continuously are presented in comparison with similar results for each block of plots on which the three-year rotation has been followed. As in Table 9, the average results for soluble phosphorus and exchangeable calcium on the unlimed plots are shown in separate columns. The average soluble phosphorus content and the average exchangeable potassium content of the plots which have been continuously in potatoes are very much higher than those for the three blocks in rotation. This is no doubt due to the fact that these elements were applied in the fertilizer each year where potatoes were grown continuously but only once every three years to the potato crop in the rotation. There was some variation between blocks in the values found for exchangeable calcium and magnesium. For the unlimed plots, the exchangeable calcium content was very low in each case and the differences were probably not significant.

DISCUSSION OF RESULTS

Although the experiment was not laid out in such a way that the results could be analysed statistically, results obtained over a period of 22 years should give a definite indication of results that can be expected under comparable conditions. It seems to be clearly indicated that the yields of potatoes when grown continuously were less than when grown in rotation. The beneficial effect of applications of manure were quite apparent and the experimental results show that, in nearly every case, a manure treatment alone gave higher yields than where the application was half manure and half commercial fertilizers. The use of nitrate of soda as a source of nitrogen was preferable to sulphate of ammonia, particularly when no manure was used. In many cases, yields on the sulphate of ammonia plots without manure were lower than on the untreated plots. Liming increased the yields of all crops and gave the greatest increases where sulphate of ammonia without manure had been applied over the 14-year period before liming was started. During the last few years when a double application of manure and fertilizer has been made to part of the plots, the results indicate that the yields of potatoes were increased and the yields of clover were improved on all plots where manure was applied, but yields of barley were not increased. However, the length of time that the double treatment has been used has been too short for any general conclusion to be reached.

The results of the determinations made on the soil samples have indicated that the applications of manure have increased the organic matter levels, the readily soluble phosphorus and the exchangeable base content. Applications of sulphate of ammonia over a long period reduced the soil reaction and exchangeable calcium. The pH values were increased on all plots where lime was applied, as were those for exchangeable calcium and readily soluble phosphorus. There is an indication that continuous cropping has reduced the organic matter below that found where the rotation was followed. Levels of soluble phosphorus and exchangeable potassium were considerably higher on the plots continuously cropped to potatoes, probably due to the more frequent application of fertilizers.

CONCLUSIONS

General conclusions from this experiment are: (1) that better yields of potatoes can be obtained when this crop is grown in rotation; (2) that manure is extremely beneficial on this soil; (3) that nitrate of soda is preferable to sulphate of ammonia as a source of nitrogen on this soil; and (4) that the use of ground limestone is beneficial.

STUDIES OF THE COMPOSITION OF DECIDUOUS FOREST TREE LEAVES BEFORE AND AFTER PARTIAL DECOMPOSITION[1]

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INTRODUCTION

Podsol soils have been intensively studied ever since the pioneer researches of Dokuchaev (9), yet the mechanism of their development is still obscure. It is generally assumed that acidic substances are responsible for the transport of bases during podsolization and many of the earlier investigators (9) stressed the importance of organic acids in this process. However, the only evidence supporting this idea was the presence of an acidic A_0 layer, as organic acids were not isolated from soil until 1913 (26). Uronic carboxyl groups have also been proposed as the agents involved in podsolization (19).

In view of the importance of podsol soils in Eastern Canada (29) an investigation of the fundamental processes responsible for their development was started at Macdonald College in 1947. The problem is being approached through a study of the acidic components leached by natural rainfall from decomposing leaves of deciduous forest trees. In connection with this work it was important to know the annual leaf-fall of the different species of trees and the nature and extent of decomposition of the leaves during weathering. These studies are described in the following sections of this paper.

EXPERIMENTAL PROCEDURE

Selection and Preparation of Sampling Areas

In September, 1947, sites were selected in the Morgan Arboretum at Macdonald College for the purpose of collecting and measuring the autumn leaf-fall of the following species of trees: American beech (*Fagus grandifolia* Ehrh.); gray birch (*Betula populifolia* Marsh.); sugar maple (*Acer saccharum* Marsh.) and poplar (*Populus grandidentata* Michx. and *P. tremuloides* Michx.).

The beech and maple stands are mature, quite dense and free of other species, situated on deep sand and glacial till, respectively. The birch areas were located in a relatively open pasture on a mixed sand and clay formation in 1947 and 1948. New sites were selected in 1949 under more dense growth on deep sand. In 1947, the poplar leaf-fall was a mixture of nearly one-third *P. tremuloides* and two-thirds *P. grandidentata*. The stand was thin and mixed with birch, maple and evergreens. New sites were selected in 1948 under a thin, mature stand of *P. grandidentata* located on imperfectly drained sand.

All undergrowth and loose, partly decomposed litter were removed from the selected areas which were then enclosed with rot-proofed canvas windscreens. Several sites were prepared under each species. The total area enclosed by windscreens was nearly 400 sq. ft. under beech and poplar, 500 sq. ft. under maple and 750 sq. ft. under birch.

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Collection of Leaf-litter

The leaves falling within the enclosed areas were collected at frequent intervals during the period of leaf-fall. Beech, maple and poplar leaves were picked up by hand. The birch leaves, collected with a hand-rake, were contaminated with a small amount of grass. Leaves of other species falling inside the screened areas were discarded. In 1947, eleven collections were made between October 9 and November 4 when leaf-fall was complete. Only in one or two instances were the fallen leaves exposed to rain before being collected. Collections were made from September 29 to November 11 in 1948. The weather was less favourable than during the previous season and an estimated 50 per cent of the leaf-litter was leached to some extent by rain before harvesting. In 1949, leaf collection began on October 1 and was completed on November 6. Only the last collection was severely leached before harvest.

The leaves of each species were put in separate bags as collected and stored in a small building in the arboretum. When leaf-fall was finished, each species was thoroughly mixed and weighed. Known quantities were exposed to weathering as described in the following section and the remainder taken to the laboratory for analysis.

Methods of Weathering Leaves

The leaves collected in 1947 were exposed to decomposition by rain and microorganisms under two different conditions.

A known quantity of leaves of each species was placed on 1/8-in. mesh wire screens, supported on trays specially designed to collect the natural rainfall percolating through the layer of leaves. Each tray was 4 ft. × 3 ft. × 10 in. with a bottom sloping towards the centre and front terminating in a pipe connected to a covered pail. The trays were set into the ground with the top about one inch above ground level. A trench was dug the required depth to hold the pails for collecting the leachates resulting from natural rainfall. Each tray was covered with 1/4-in. mesh wire screens to exclude small animals, wind-blown leaves, etc. All surfaces of screens, trays and pails were treated with asphalt paint to inhibit corrosion. The leaves were exposed in the trays for 11 months.

To compare the degree of decomposition of leaves in trays with that of leaves lying over soil, a known amount of each species was exposed to weathering for 10 months on 1/8-in. mesh wire screens (4 ft. × 3 ft.) in contact with the ground and held in place by canvas windscreens. These were located on a single soil type in the vicinity of the trays.

METHODS OF ANALYSIS

Analysis of Leaf Material

Samples of the freshly fallen leaves and the residues from the trays and ground-screens were air-dried in the laboratory, weighed, thoroughly mixed and a representative portion of each ground in a Wiley Mill so that all of the material would pass through a 20-mesh sieve. The chemical determinations were made on representative sub-samples of this ground material.

Moisture was calculated as the loss in weight when two-gram samples were dried to constant weight in an electric oven at 105° C.

Ash was found by igniting weighed samples in porcelain crucibles in a muffle at 600° C.

Total nitrogen was determined by the micro-Kjeldahl method (20).

Lignin determinations were made by the 72 per cent sulphuric acid method as described by Ritter and co-workers (25), after removal of extractives and pretreating with one per cent hydrochloric acid.

"Extractive-free" material for subsequent holocellulose determinations was prepared by a modification of the procedure employed for wood (30). Weighed samples of ground leaf material were transferred to fritted glass extraction thimbles (C porosity) and extracted in a Soxhlet for 16 hours with alcohol-benzene solution (32 parts by weight of alcohol and 68 parts by weight of benzene). The residue was washed several times with alcohol on a Buchner, using light suction, and allowed to air-dry until free of alcohol. It was then refluxed for three hours with distilled water (150 ml./g.), filtered, washed several times with hot water, and air-dried. Quantitative estimation of the alcohol-benzene and hot water soluble material was obtained using five-gram samples. Large batches of "extractive-free" material were prepared using 15-g. samples for the first extraction and combining the contents of two thimbles for the boiling water digestion. Any sand contaminating the leaf material remained in the digestion flask when the extracted residue was carefully transferred to a Buchner by successive washings with hot distilled water. It was collected in a tared Gooch crucible, ignited at 600° C. and weighed. The mean of six 30-g. samples was calculated as per cent sand. All results in this paper are reported on the basis of the sand-free materials.

The "extractive-free" material was used for the determination of holocellulose, employing the acid chlorite method of Wise, Murphy and D'Addieco (35).

Alpha cellulose was determined as described by Ritter (24) on samples of air-dried holocellulose.

Analysis of Leachates

The leachates were collected from the trays at each rainfall during the period of weathering except for the winter months. They were filtered to remove any suspended matter and the volumes recorded. Titratable acidity or alkalinity was determined by titrating a suitable aliquot to pH 7 with 0.01 N hydrochloric acid or sodium hydroxide; pH was measured with a potentiometer using a glass electrode. Non-volatile solids and ash were found by the methods used for potable water (20). Ash alkalinity was determined by the method described by Purves (22).

RESULTS AND DISCUSSION

The Amount of Leaf-Fall from Hardwoods

The leaf-fall of beech, birch, maple and poplar trees was measured for three successive years. The results are given in Table I.

The yield was highest from maple followed by beech, birch and poplar. The leaf-fall varies considerably from year to year, particularly under birch and poplar, indicating these two species may be more susceptible to variations in climatic conditions than maple and beech.

TABLE 1.—ANNUAL LEAF-FALL OF FOUR SPECIES OF DECIDUOUS FOREST TREES

(Values represent pounds of dry matter per acre)

Species	1947	1948	1949	Average
Maple	2690	3116	3225	3010
Beech	1689	2096	2043	1943
Birch	1136	2341	1032	1503
Poplar	1421*	2044	1002	1489

* Mixture of *P. grandidentata* and *P. tremuloides*.

Except for maple, the leaf-fall in the Morgan Arboretum appears to be less abundant than in New York State. Chandler (5) found the fall of hardwood leaf litter ranged from 2425–3020 lb. of dry matter per acre on several soil types in 1940. The average fall of litter from conifers is of the same magnitude as from hardwoods. Thus a mixed stand of white and Norway pine in southern New England gave an annual yield of 2000–3000 lb. of litter per acre (17).

According to Lutz and Chandler (11) tree leaves are the largest single source of forest soil organic matter. Watson (34) quotes Ebermayer who found the annual leaf-fall in a mature beech forest nearly equivalent to the fall of wood.

The Composition of Freshly Fallen Leaves

The mineral composition of forest litter has received considerable attention (2, 5, 6, 10, 14, 16, 28), but there is little information available regarding the organic composition of leaves of different forest trees. In Table 2 are recorded the analyses of freshly fallen leaves collected in 1947.

Maple leaves have the highest ash content of the four species. Lutz and Chandler (11) give the average ash content of maple leaves as 11.85 per cent. Of the common hardwoods, maple leaves generally have the highest ash content. Melin (15) found the ash content of beech leaves to be

TABLE 2.—THE COMPOSITION OF MATURE LEAVES FROM FOUR SPECIES OF DECIDUOUS TREES

(Values represent percentages based on dry matter)

Fraction	Beech	Birch	Maple	Poplar*
Ash	9.44	7.21	12.33	9.41
Nitrogen	0.81	1.12	0.83	0.84
Alcohol-benzene soluble	16.18	18.30	20.42	19.54
Hot water soluble	13.63	21.48	23.68	22.03
Apparent lignin, ash free	24.03	21.87	15.49	20.00
Holocellulose	35.15	24.89	26.82	27.09
Alpha cellulose			18.06	18.91
Hemicellulose, crude			8.76	8.18

* Mixture of *P. grandidentata* and *P. tremuloides*.

9.36 per cent although the average of several reported values is 7.37 per cent (11). Leaves of *P. grandidentata* and *P. populifolia* had ash contents of 3.85 and 4.07 per cent, respectively (15). Numerous investigations have shown that the ash content of forest tree leaves may vary considerably according to species, age of the leaves and composition of the soil.

The nitrogen contents of the leaves as found in the present study are in general agreement with those reported in the literature (11, 5, 10).

Melin (15) determined the proximate composition of several species of mature freshly fallen leaves using Waksman's modified method (31, III). Three of the species, beech, maple and poplar were used in the present study and although different analytical methods were employed it is of interest to compare the results. Reasonably good agreement was obtained for the carbohydrate and lignin fractions, except for the lignin content of poplar leaves. Melin (15) reports a value of 32.69 per cent as compared to 20.00 per cent found in the present study. A higher content of water soluble material and substances soluble in organic solvents was found in the present work as compared to those found by Melin, except for the water soluble content of maple leaves which agrees fairly well. The differences in the analyses may be partly due to the fact that the leaves analysed by Melin were not representative of the total leaf-fall. In both studies, the species appear in the same order with regard to content of "extractives", i.e., maple, poplar and beech.

Decomposition of Freshly Fallen Leaves

Visual observations during the summer of 1948 indicated that maple and birch leaves decomposed more rapidly than those of poplar and beech. These observations are confirmed by the data given in Table 3 for leaves collected in the autum of 1947.

Birch and maple leaves decomposed nearly the same amount on the trays and over the soil. Beech and poplar leaves weathered more extensively in contact with the soil than in the trays.

The above experiment was repeated using leaf-litter collected during the autumn of 1948 and exposing an amount of each species equivalent to 10 times the fresh weight collected per 12 sq. ft. of ground surface. The

TABLE 3.—LOSS IN WEIGHT OF LEAVES EXPOSED TO WEATHERING IN TRAYS AND IN CONTACT WITH THE SOIL

(Basis: 100 g. oven-dry unweathered leaves)

Species	Trays*		Soil*	
	Original weight (g)	Loss (%)	Original weight (g)	Loss (%)
Beech	3218	18.2	7012	30.3
Birch	6022	38.2	8184	41.6
Maple	3581	42.7	7209	41.0
Poplar†	2996	24.5	5519	35.1

* After 11 months on trays and 10 months over soil, respectively.
† Mixture of *P. grandidentata and P. tremuloides.*

amounts exposed in the trays and in contact with the soil ranged from 2560 g. of poplar to 3899 g. of maple leaves. Under these conditions decomposition in every case was considerably greater in the trays than over soil, although less than the previous year. In both experiments the loss in weight was nearly the same for maple and birch, less for poplar, and beech leaves were most resistant to decomposition.

It was noted that the leaves exposed in the trays and on the ground in 1949 dried out more rapidly following a rain than in 1948, presumably because of the larger quantity of leaves on the screens in 1948. Also, the tray residues contained more moisture than the ground residues when sampled in September, 1949, the soil apparently acting like a blotter. The total precipitation from April to September at Macdonald College was four inches higher in 1949 than in the previous year; however all of this excess fell in April and September. Precipitation during the summer months was very nearly the same in both years. Bartholomew and Norman (3), and Waksman and Purvis (33) have emphasized the importance of moisture in the microbiological decomposition of organic materials. In the present study, it appears that the rate of decomposition is markedly influenced by the moisture content which in turn is affected by the quantity of material, the conditions of exposure and climatic conditions.

It is interesting to compare these results with those obtained by other workers. Lunt (10) obtained losses of 2.7 and 7.0 per cent respectively, on exposing mature beech and maple leaves to natural weathering for seven weeks. Gustafson (8) found that freshly fallen maple leaves lost 30 per cent of their dry matter during exposure on the ground in a mixed stand of hardwoods for one year. Melin (15), and Broadfoot and Pierre (4) studied the decomposition of various species of forest leaves under controlled conditions in the laboratory. Beech leaves were most resistant to decomposition, birch was readily attacked, while maple and poplar were intermediate. The agreement between the results of these investigators and those obtained in the present study is significant, particularly when it is considered that the conditions were quite different in the various investigations.

Changes in the Composition of Leaves during Weathering

The decomposition of plant materials is influenced by many factors, such as the nature of the fresh material, the environmental conditions and the microorganisms and fauna concerned. Many investigators (12, 13, 15, 18, 23, 31, 34), have shown that there is a rapid loss of water soluble substances such as sugars, starches, and nitrogenous compounds in the initial stages of decomposition. The components of the cell wall are attacked less rapidly. Cellulose may disappear completely after a period of time, but lignin is relatively resistant to microbiological attack.

The general group of hemicelluloses appear to be attacked fairly rapidly, particularly in the early stages of decomposition. However, it must be emphasized that these conclusions are based mainly on results obtained by the proximate analysis of plant materials and soil. This method of study does not distinguish between living and dead organic matter or between plant remains and organic matter of microbial origin. Over 30 per cent of the organic matter in fertile soils may be microorganic, consisting of nitrogenous compounds, humus-like substances and complex carbohydrates (7). Thus it is evident that the method of proximate analysis provides a very incomplete picture of the changes which take place in decomposing organic matter.

The composition of the partially decomposed tray residues, resulting from the weathering of leaves collected in 1947, is given in Table 4. Changes in composition, calculated as percentages of the amounts of the different fractions present in 100 g. of oven-dry original leaves (see Table 2), are recorded in Table 5.

The greatest losses have occurred in the alcohol-benzene soluble, hot water soluble, ash and holocellulose fractions of all species. The decrease in the holocellulose fraction of poplar appears to be due almost entirely to loss of alpha cellulose. The crude hemicellulose (holocellulose-alpha cellulose) content of partially decomposed poplar leaves is only slightly less than in the unweathered leaves (see Tables 2 and 4). The loss of hemicelluloses was probably more extensive than indicated by the data since the yield of holocellulose was always less than the theoretical quantity, pre-

TABLE 4.—THE COMPOSITION OF LEAVES COLLECTED IN 1947 AFTER ELEVEN MONTHS WEATHERING IN TRAYS

(Basis: 100 g. of oven-dry unweathered leaves)

Fraction	Beech	Birch	Maple	Poplar
Ash	7.50	3.47	5.52	6.72
Nitrogen	0.92	1.18	0.69	0.79
Alcohol-benzene soluble	7.00	6.91	5.82	8.95
Hot water soluble	7.33	6.16	10.41	7.12
Apparent lignin, ash free	25.72	24.32	13.57	27.92
Holocellulose	28.58	12.44	15.40	17.46
Alpha cellulose	—	—	8.95	9.38
Hemicellulose, crude	—	—	6.45	0.08

TABLE 5.—PERCENTAGE CHANGE IN THE COMPOSITION OF LEAVES DURING WEATHERING

(Basis: 100 g. of oven-dry unweathered leaves)

Fraction	Beech	Birch	Maple	Poplar
Ash	−20.5	−51.8	−55.2	−28.5
Nitrogen	+12.3	+ 5.3	−16.8	− 5.9
Alcohol-benzene soluble	−56.7	−62.2	−71.4	−54.1
Hot water soluble	−46.2	−71.3	−56.1	−67.6
Apparent lignin, ash free	+ 7.1	+10.1	−12.3	+39.6
Holocellulose	−18.6	−50.0	−42.5	−35.5
Alpha cellulose	—	—	−50.4	−50.3
Hemicellulose, crude	—	—	−26.3	− 1.2

sumably due to hydrolysis of some of the hemicelluloses during acid chlorite digestion. Adams and Castagne (1) obtained evidence of hydrolysis of hemicelluloses when preparing holocellulose samples of cereal straws. Similar results were experienced in the preparation of wood holocellulose (36). The theoretical holocellulose content of a plant material is obtained by subtracting from 100, the sum of the percentages of ash, lignin and protein (N × 6.25) in the oven-dry extractive-free material. The percentage holocellulose in the unextracted original tissue may then be calculated using a factor determined by the analytical results.

The increase in the apparent lignin content of poplar leaves, and to a less extent in birch and beech, as a result of decomposition emphasizes the inadequacy of the method of determining lignin. Waksman (32) cites several papers which suggest that lignin is decomposed to some extent by certain microorganisms. On the other hand there is evidence that certain dark-colored filamentous fungi, commonly inhabiting soil and decaying plant material, may contain substances insoluble in 72 per cent sulphuric acid (21). The actual structure of lignin is unknown and as pointed out by Norman (19), there may be many lignins just as there are many proteins. Thus, it is quite evident that insolubility in 72 per cent sulphuric acid is a very empirical measure of lignin, especially in a complex system such as decomposing organic matter.

The composition of a plant material is one of several factors which influence the rate of decomposition. In general, a low content of water soluble substances and nitrogen, accompanied by a relatively high amount of lignin is conducive to slow decomposition (31, 32). Thus the slow rate of decomposition of beech leaves as compared to that of maple would be expected on the basis of their composition (see Table 2). Birch leaves contained more nitrogen than poplar which likely influenced the rate of decomposition of the former.

Characteristics of Leachates

The characteristics of the natural rainwater leachates obtained from the trays during eleven months' exposure of leaves collected in the autumn of 1947 are summarized in Table 6.

TABLE 6.—CHARACTERISTICS OF LEACHATES FROM LEAVES DECOMPOSING ON TRAYS DURING 1947–48

(Basis: Total volume of leachates collected)

Analysis	Beech	Birch	Maple	Poplar
Non-volatile solids (g.)	66.0	257.4	204.5	118.1
Ash (g.)	27.7	85.9	41.5	32.4
Titratable acidity (m.e.)	53.5	−24.7	261.7	50.8
Ash alkalinity (m.e.)	150.4	927.9	493.7	318.8
Total acidity (m.e.)	203.9	903.2	755.4	369.6
Ash alkalinity/ash	5	11	12	10

The amount of non-volatile solids and ash removed by leaching was greatest from birch, followed by maple, poplar and beech. The degree of decomposition based on loss in weight followed nearly the same order of species (see Table 3). Maple leachates had the highest titratable acidity, followed in descending order by beech, poplar and birch. Assuming that the bases were neutralizing acids, the total acidity was calculated by adding together the values for titratable acidity and ash alkalinity. Birch and maple leachates have high total acidities compared to those of poplar and beech.

The leachates of each species became progressively more alkaline with time. This is in agreement with the findings of Stepanov (27). Maple leachate was consistently the most acidic, with a minimum pH of 4.4 and a maximum of 6.5. Birch leachates collected in the late fall and early spring were acidic, but the pH increased to 7.0 by May 1 and remained alkaline throughout the season, reaching a maximum value of 7.7. Beech and poplar leachates had pH values between 5.5 and 5.8 in the fall, increasing during the following summer to neutrality or slight alkalinity. Poplar leachates were slightly less acidic than beech in most cases.

The above results were confirmed by the analysis of leachates obtained during 1948–49, from trays containing leaves collected in the fall of 1948 (see p. 460). The results appear in Table 7.

TABLE 7.—CHARACTERISTICS OF LEACHATES FROM LEAVES DECOMPOSING ON TRAYS DURING 1948–49

(Basis: total volume of leachates collected)

Analysis	Beech	Birch	Maple	Poplar
Non-volatile solids (g.)	48.6	87.0	270.6	82.6
Ash (g.)	16.8	22.7	65.4	29.2
Titratable acidity (m.e.)	21.6	−7.9	261.5	−15.4
Ash alkalinity (m.e.)	114.9	290.0	632.3	302.0
Total acidity (m.e.)	136.5	282.1	893.8	286.6
Ash alkalinity/ash	6	13	10	10

There are some differences apparently due to the amount of leaves exposed to weathering. The low content of solids in the birch leachates is surprising, particularly as the degree of decomposition of birch and maple leaves was nearly the same, 27.5 and 27.2 per cent respectively. The value obtained for alkalinity of ash may vary as a result of several factors, such as the ratio of monovalent to divalent cations and the proportion of basic to acidic oxides in the ash. However, the relative constancy of the ratio of ash alkalinity to ash for all species in both experiments suggests little change in the composition of the ash of the leaves in the two successive years. Further, the ash composition of birch, maple and poplar leachates may be essentially the same.

SUMMARY

The leaf-fall of four species of deciduous forest trees was determined for three successive years. The average yearly leaf-fall of each species in pounds of dry matter per acre was as follows: maple—3010; beech—1943; birch—1503; poplar—1489.

The proximate composition of the freshly fallen leaves of each species was determined. Maple leaves had the highest content of ash and extractives, and the lowest lignin content. Beech leaves had the lowest content of extractives and the largest amount of lignin. Birch and poplar leaves were intermediate. The holocellulose content was highest in beech leaves, followed by poplar, maple and birch.

Maple and birch leaves decomposed at twice the rate of beech leaves when exposed to weathering in trays. The differences were not so marked in the case of leaves lying in contact with the soil. Poplar leaves decomposed at an intermediate rate.

The rate of decomposition was related to leaf composition, particularly in the case of beech and maple leaves. The extent of decomposition under the conditions employed was markedly influenced by the moisture content of the decomposing material.

Ash, extractives, and alpha cellulose were quite readily removed during decomposition. Lignin and hemicelluloses were relatively resistant to decomposition. Considerable variation existed between species.

Natural rainwater leachates from maple leaves were strongly acidic throughout the season as shown by pH and titratable acidity. Leachates of birch leaves were the least acidic, while beech and poplar leachates were intermediate. In all cases the leachates became progressively more alkaline as the time and extent of decomposition increased.

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DIGESTIBILITY STUDIES WITH SWINE

IV. ASSOCIATIVE DIGESTIBILITY BETWEEN BARLEY AND TANKAGE[1]

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The Division of Animal Husbandry of the Experimental Farms Service has collaborated in the development of an Advanced Registry Feeding Ration to provide a standard ration for the testing of pigs at the various Advanced Registry Feeding Stations across Canada. It consists of the basal grains, barley, wheat and oats, plus a protein mineral supplement. The ration up to 1949 was as follows:

Basal Feed Mixtures

From 70 days to 100 lb.	Per cent	*100 lb. to 200 lb.*	Per cent
Barley	50	Barley	60
Wheat	20	Wheat	30
Oats	30	Oats	10

To this was added a protein-mineral supplement. The level of this supplement was 15 per cent of the total ration for swine of the 70 day to 100 lb. group and 6 per cent of the total ration for swine of the 100 lb. to 200 lb. group.

The protein-mineral supplement was made up as follows:

	Per cent
Tankage	50
Linseed Oil Meal	25
Fishmeal	15
Iodized Salt	5
Limestone	5

It has been found that on this ration, particularly when self-fed, pigs are often over-finished and suffer a cut in market value.

Consideration has been given to lowering the amount of barley, increasing the amount of oats and changing the amount of protein-mineral supplement in this ration.

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TABLE 1.—RATIONS EMPLOYED IN DIGESTIBILITY TRIALS WITH SWINE

Ration No.	Constitution of Ration		Numbers given in Tabulation
	Barley %	Tankage %	
1	10	90	T9
2	80	20	T2
3	40	60	T6
4	20	80	T8
5	100	0	B
6	60	40	T4

TABLE 2.—SUMMARY OF COEFFICIENTS OF DIGESTIBILITY OF INDIVIDUAL NUTRIENTS IN THE TOTAL RATIONS. (COEFFICIENTS IN PER CENT, RATION NUMBERS IN BRACKETS GIVEN ONLY IN FIRST SQUARE, MISSING VALUES IN SQUARE BRACKETS)

Nutrient	Period No.	Coefficients of digestibility in per cent for following animals*						Means
		11	12	13	14	15	16	
Dry matter	1	[59(T9)]	78(T2)	85(B)	72(T4)	63(T8)	66(T6)	72.8
	2	69(T4)	61(T6)	74(T2)	56(T8)	56(T9)	81(B)	66.2
	3	74(T2)	82(B)	62(T8)	[55(T9)]	61(T6)	67(T4)	69.2
	4	60(T6)	52(T9)	67(T4)	75(T2)	80(B)	56(T8)	65.0
	5	80(B)	53(T8)	52(T9)	61(T6)	67(T4)	73(T2)	64.3
	6	[58(T8)]	69(T4)	62(T6)	80(B)	73(T2)	55(T9)	67.8
	Means	70.8	65.8	67.0	68.8	66.7	66.3	67.3
Organic matter	1	77	83	86	80	77	78	80.8
	2	78	75	80	74	75	82	77.3
	3	82	83	76	75	74	77	78.4
	4	73	71	77	80	81	74	76.0
	5	81	70	71	76	77	79	75.7
	6	75	79	75	82	80	75	78.2
	Means	78.5	76.8	77.5	78.4	77.3	77.5	
Nitrogen	1	86	86	88	85	86	82	85.4
	2	81	85	83	84	86	82	83.5
	3	81	84	85	85	82	80	82.4
	4	82	84	82	83	80	82	82.2
	5	80	81	83	82	80	82	81.3
	6	82	84	82	82	81	83	82.4
	Means	81.0	84.0	83.8	83.2	82.5	81.8	
Ether extract	1	97	91	61	95	96	96	87.8
	2	96	96	90	96	97	68	90.5
	3	95	70	97	101	96	94	90.4
	4	96	96	94	93	44	96	86.4
	5	52	96	96	96	94	91	87.5
	6	94	96	91	56	91	96	86.0
	Means	84.8	90.8	88.2	87.2	86.3	90.2	
T.D.N. in dry matter	1	65	79	84	74	65	69	74.2
	2	72	68	76	64	64	81	70.8
	3	79	82	65	64	67	71	72.8
	4	66	61	72	77	80	64	70.0
	5	80	61	60	68	71	75	69.2
	6	63	72	66	80	76	63	71.4
	Means	74.3	70.5	70.5	72.6	70.5	70.5	
Energy	1	77	80	85	77	76	77	79.0
	2	76	72	76	70	75	81	75.0
	3	80	82	76	75	73	75	77.2
	4	70	70	74	79	80	73	74.3
	5	79	66	69	70	74	76	72.3
	6	72	75	71	80	77	75	75.6
	Means	76.3	74.2	75.2	75.2	75.8	76.2	

* Individual values calculated to one decimal place but tabulated in round numbers to conform with editorial policy.

The Division of Chemistry is carrying out a series of digestion trials with swine to determine if variations in the proportions of basal grains and in the proportion of protein supplements to the grain mixture will affect their digestibility. In view of the contemplated changes in the Advanced Registry Feeding Station ration, it was considered advisable to use the Advanced Registry Ration for further studies of the associative digestibility between swine feeds.

Three experiments were planned and completed as follows:

(1) Associative digestibility between barley and tankage.

(2) Associative digestibility between the three basal grains, barley, wheat and oats, with the protein-mineral supplements maintained constant.

(3) Associative digestibility between the protein-mineral supplement and the grains with the ratio of the three grains to each other kept constant.

The results of the first of these are given in the present paper. The results of the other two will appear in subsequent papers.

PROCEDURE

Digestibility trials were carried out on rations of No. 1 Western Feed Barley and tankage. These are listed in Table 1. The chemical compositions are given in Table 8 of the appendix.

Six purebred Yorkshire barrows were used. Their weights throughout the experiment are given in Table 9 in the appendix. The six rations were fed to the six animals during six periods in the form of a 6 x 6 randomized Latin square. Each period consisted of 21 days—4 days change-over, 7 days preliminary and 10 days collection. As far as possible the animals were fed according to feeding standards. The feed was given in the form of a slop. Supplements of feeding oil, iodized salt, nicotinic acid and pantothenate were given. No calcium or phosphorous was given. The barley was cracked.

PROGRESS OF EXPERIMENT

There were feed refusals in period 1 on the 90 per cent tankage ration, in period 3 on the 90 per cent tankage ration and in period 6 on the 80 per cent tankage ration. The results from these three digestion trials were not used. In period 4, the 90 per cent tankage ration was being refused in the second half of the 10-day collection period. Therefore, the results for only the first five days were used. In period 5, the animal on barley did not eat too well during the change-over period, therefore the preliminary period was lengthened. This reduced the collection period to six days. Apart from these cases the trials proceeded satisfactorily. There were, then, three missing values in the Latin square.

RESULTS

A summary of the coefficients of digestibility is given in Tables 2 and 3. The data have been arranged in 6 x 6 Latin squares. The missing values have been included in brackets.

The analysis of variance is given in Table 4.

TABLE 3.—MEAN COEFFICIENTS OF DIGESTIBILITY OF INDIVIDUAL NUTRIENTS IN THE TOTAL RATIONS ARRANGED BY RATIONS

Nutrient	Coefficients of digestibility, in per cent, of following rations					
	T9	T8	T6	T4	T2	B
Dry matter	53.7	58.0	61.8	68.5	74.5	81.3
Organic matter	73.0	74.2	75.2	78.0	80.7	82.5
Nitrogen	84.0	83.6	82.5	82.0	82.7	82.7
Ether extract	96.3	96.2	95.2	94.8	91.8	58.5
T.D.N. in dry matter	62.0	63.8	67.3	72.0	77.0	81.2
Energy	72.3	72.2	72.2	75.2	78.0	81.2

TABLE 4.—ANALYSIS OF VARIANCE OF DATA IN TABLES 2 AND 3

Nutrient	Variance due to	Designation D/F		Sums of Squares	Variance	F*
Dry matter	Total	Original	32	3,013		
	Error	Complete	17	43	2.53	
	Per. + An. + Rat.	Diff.	15	2,970		
	Per. + An.	#	10	398	39.80	†
	Ration	Diff.	5	2,572	514.40	†
Organic matter	Total	Original	32	505		
	Error	Complete	17	60	3.53	
	Per. + An. + Rat.	Diff.	15	445		
	Per. + An.	#	10	116	11.60	3.29
	Ration	Diff.	5	329	65.80	†
Nitrogen	Total	Original	32	129		
	Error	Complete	17	31	1.82	
	Per. + An. + Rat.	Diff.	15	98		
	Per. + An.	#	10	83	8.30	4.56
	Ration	Diff.	5	15	3.00	1.65
Ether extract	Total	Original	32	7,038		
	Error	Complete	17	333	19.59	
	Per. + An. + Rat.	Diff.	15	6,705		
	Per. + An.	#	10	258	25.80	1.32
	Ration	Diff.	5	6,447	1,289.40	†
T.D.N. in dry matter	Total	Original	32	1,565		
	Error	Complete	17	22	1.29	
	Per. + An. + Rat.	Diff.	15	1,543		
	Per. + An.	#	10	176	17.60	†
	Ration	Diff.	5	1,367	273.40	†
Energy	Total	Original	32	580		
	Error	Complete	17	51	3.00	
	Per. + An. + Rat.	Diff.	15	529		
	Per. An.	#	10	178	17.80	5.93
	Ration	Diff.	5	351	70.20	†

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* Nec. F. for $N_1 = 10$, $N_2 = 17$ at P of 0.05 = 2.46.
Nec. F. for $N_1 = 5$, $N_2 = 17$ at P of 0.05 = 2.81.

# Obtained from analysis of variance of data when arranged by animals and periods, ignoring rations and using Yates' (5) method for incomplete blocks as outlined by Geulden (3).

† Obviously significant.

The statistical methods for these operations were as follows. The missing values were estimated by Yates' method as outlined by Cochran and Cox (1). The total sums of squares were calculated from the original values and the error sums of squares from the complete values. The difference gave the sums of squares due to period, animal and ration. To determine the variance contributed by the periods and animals, a suggestion by Yates (6), adapted by Goulden (personal communication) was used. The data were arranged by animals and periods, ignoring ration effects. Missing values and subsequent analyses of variance were calculated by Yates' (6) method as outlined by Goulden (3) for incomplete randomized blocks. From this the sums of squares for periods and animals were determined. It will be seen from Table 4 that a significant variance was contributed by animals plus periods. The standard errors for single

TABLE 5.—STANDARD ERRORS OF COEFFICIENTS OF DIGESTIBILITY OF SINGLE MEANS FOR INDIVIDUAL NUTRIENTS OF RATIONS IN TABLE 3

No. of missing values in ration mean	Standard errors of coefficients of digestibility of following nutrients					
	Dry matter	Organic matter	Nitrogen	Ether extract	T.D.N. in dry matter	Energy
None	0.65	0.77	0.55	1.81	0.46	0.71
One	0.80	0.94	0.68	2.21	0.57	0.87

TABLE 6.—COMPARISON OF MEANS OF OBSERVED COEFFICIENTS OF DIGESTIBILITY WITH THE THEORETICAL VALUES OF RATIONS CONTAINING 20, 40, 60 AND 80 PER CENT OF TANKAGE, RESPECTIVELY

Ration		Designation	Mean coefficients of digestibility in per cent of following nutrients					T.D N. in per cent of dry matter
Barley %	Tankage %		Dry matter	Organic matter	Nitrogen	Ether extract	Gross energy	
20	80	Observed	58.0	74.3	83.3	95.8	72.2	63.7
		Theoretical	57.8	75.3	84.4	96.4	74.4	64.6
		Difference	+0.2	−1.0	−1.1	−0.6	−2.2	−0.9
		"t" *	0.25	1.06	1.62	0.27	2.53	1.58
40	60	Observed	61.8	75.2	82.5	95.2	72.2	67.3
		Theoretical	63.5	77.5	84.2	94.2	76.2	68.6
		Difference	−1.7	−2.3	−1.7	+1.0	−4.0	−1.3
		"t" *	2.61	2.98	3.09	0.56	5.63	2.83
60	40	Observed	68.5	78.0	82.0	94.8	75.2	72.0
		Theoretical	69.3	79.4	83.9	90.5	77.9	72.7
		Difference	−0.8	−1.4	−1.9	+4.3	−2.7	−0.7
		"t" *	1.23	1.82	3.45	2.38	3.80	1.52
80	20	Observed	74.5	80.7	82.7	91.8	78.0	77.0
		Theoretical	75.2	81.0	83.5	83.0	79.6	76.9
		Difference	−0.7	−0.3	−0.8	+8.8	−1.6	+0.1
		"t" *	1.08	0.39	1.45	4.86	2.25	0.22

* Necessary "t" for N = 17, P of 0.05 = 2.11.
Necessary "t" for N = 17, P of 0.01 = 2.90.

TABLE 7.—MEAN COEFFICIENTS OF DIGESTIBILITY AND TOTAL DIGESTIBLE NUTRIENTS IN DRY MATTER OF TANKAGE CALCULATED FROM THE MIXED RATIONS WITH BARLEY. (MEANS OF SIX VALUES)

Ration		Coefficients of digestibility in per cent of following nutrients in tankage					T.D.N. in per cent of dry matter
Barley	Tankage	Dry matter	Organic matter	Nitrogen	Ether extract	Energy	
%	%						
10	90*	52	72	84	97	72	61
20	80*	53	71	84	97	70	60
40	60	50	68	83	99	66	58
60	40	50	68	82	103	63	58
80	20	49	69	82	112	64	60

* Including missing values.

means and for the differences between two means were determined by a method outlined by Cochran and Cox (1). Period 1 gave results which were higher than those of the other periods.

To determine whether or not there were any associative effects the following procedure was adopted. From the 90 per cent tankage ration the coefficients of digestibility of tankage were calculated. Estimates of missing values were included. Using the means of these and the means of the coefficients of barley as base values, theoretical coefficients of digestibility of the remaining rations were estimated. These were compared with the means of the observed values. If the tankage in the 90 per cent ration had different coefficients than in the other rations or if the tankage in the other rations affected their digestibility, then, this would be evident from the differences between the observed and theoretical values for any one ration. Theoretical values were taken as zero values. The differences between these and the observed ones were treated according to Fisher's method for unique samples (2). The standard errors of the single means of the coefficients of digestibilities of the individual nutrients were calculated from the analysis of variance in Table 4, as explained previously. These are given in Table 5.

The comparison of the mean observed values with the theoretical ones is given in Table 6. The results for dry matter, organic matter and T.D.N. in the dry matter will first be considered. For rations containing 80, 60 and 20 per cent tankage, the differences between the observed and theoretical values were small, more than half of them being less than 0.5 per cent. They were not statistically significant. That means that there were no associative effects as far as these nutrients were concerned in rations containing, respectively, 90, 80, 40 and 20 per cent tankage. In the ration containing 60 per cent tankage, the observed values for dry matter and organic matter were 2 per cent lower and for the T.D.N. one per cent lower than the theoretical. These differences were statistically significant.

With the ether extract the differences progressively increased as the tankage in the ration decreased. At 40 per cent and 20 per cent levels of tankage they were large and significant. This may be due, at least in part, to an artefact. The ether extract, in barley, is low—about 1.8 per cent. Any ether extract in the feces of body origin would greatly affect the apparent coefficient of digestibility of the ether extract of barley. The fatty acids in barley fat are composed of approximately 44 per cent linoleic acid, 27 per cent oleic, 7 per cent palmitic, 3 per cent stearic and 0.4 per cent linolenic. This mixture should be highly digestible. The apparent coefficient of digestibility in this experiment was 58 per cent. The per cent ether extract in tankage was over ten. If the "true" value for the barley were higher than the apparent, then it would become more and more apparent in the theoretical values as the barley increased and the tankage decreased.

With regard to the energy the observed values were from two to four per cent lower than the theoretical ones. These results were not in agreement with those for the T.D.N. Feces from animals receiving high tankage rations were very high in ash. From 40 to 60 per cent was found. The caloric value per gram of organic matter was highest in the high ash feces and lowest in the feces from animals which received either barley alone or small amounts of tankage. The chemical composition revealed no reason for this difference. It was concluded that the high ash in some way interfered with the caloric determinations. The possibility was considered that calcium soaps might be formed. These would remain undigested and pass into the feces. The soaps are insoluble in fat solvents and would not appear in the ether extract fraction. To test this idea, feces from rations containing varying quantities of tankage were extracted by the method of Wood and Simpson (5). The principle of the method is acid hydrolysis, followed by extraction.

With rations containing 90, 60, 40 and 20 per cent of tankage respecttively, the fat by the acid hydrolysis method was, respectively, in round figures, 12 per cent, 6 per cent, 5 per cent and 3 per cent. By the usual petroleum ether extraction method, the fat was respectively, in round figures, 0.5, 0.6, 0.7 and 1.4 per cent. When conventional caloric values were applied to the protein, carbohydrates and fat obtained by the hydrolysis method, the caloric value was similar to that obtained in the bomb calorimeter. The influence of rations containing a high content of calcium on the digestibility of fats is being studied in an independent investigation.

With nitrogen there were no statistical differences for rations containing 80 and 20 per cent tankage. In the rations containing 60 and 40 per cent tankage the observed values were between one and two per cent lower than the theoretical. These differences were significant. The mean coefficients of digestibility of tankage calculated from the mixed rations are shown in Table 7. It will be observed that for the dry matter, organic matter, nitrogen and T.D.N. in the dry matter there was little difference between the values for tankage in the various rations. The values for the energy were lower and for the ether extract higher in the rations containing smaller quantities of tankage. These points were dealt with above.

This discussion has shown a comparison between the coefficients of digestibility and the T.D.N. in the dry matter of barley and tankage in the 90 per cent tankage ration and the same values in the 80, 60, 40 and 20 per cent tankage rations. Certain variations have been noted. It is concluded that, as a general rule, when barley and tankage are mixed together in widely varying proportions in swine rations there are no associative digestibility effects.

SUMMARY AND CONCLUSIONS

1. Six pure bred Yorkshire barrows were fed five rations of barley and tankage and one of barley alone.

2. The tankage was added to the rations in proportions of 90, 80, 60, 40 and 20 per cent of the mixture.

3. Digestibility trials were carried out.

4. Statistical procedures were described by means of which the digestibilities of the rations could be compared with theoretical ones representing the absence of associative digestibility effects.

5. The high ash content of the tankage appeared to interfere with the digestibility of the fat of that feed.

6. This point will be studied in a separate investigation.

7. It was concluded, on the basis of these comparisons, that as a general rule, there were no associative digestibility effects between barley and tankage.

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APPENDIX

TABLE 8.—CHEMICAL COMPOSITION OF FEEDSTUFFS. (MEANS OF SIX VALUES)

Feedstuff	Original dry matter	Composition of Dry Matter					
		Ash	Protein*	Ether extract	Crude fibre	Nitrogen-free extract	Energy Therms per kilogram
	%	%	%	%	%	%	
Barley	87.28	2.99	14.36	1.77	6.48	74.40	4.55
Tankage	92.17	35.03	47.24	11.08	2.31	4.34	3.85

* Protein factors: Barley—N x 5.83 (4) Tankage—N x 6.25.

TABLE 9.—LIVE WEIGHTS OF ANIMALS

Animal No.	Weight in kilograms in following periods*						Gains	
	1	2	3	4	5	6	Total	Daily‡
11	—	40	51	63	67	—	—	—
12	49	55	72	75	79	83	34	0.26
13	49	60	68	79	79	96	47	0.36
14	37	40	—	63	66	93	56	0.42
15	36	42	52	70	86	130	67	0.51
16	48	60	71	83	96	101	53	0.40
Means	44	50	63	72	79	95	51	0.39

* The weights for each period are the average of single weights before and after the collection sub-period.

‡ Length of trial from the middle date of the first period to the middle date of the sixth period = 132 days.

DIGESTIBILITY STUDIES WITH SWINE

V. ASSOCIATIVE DIGESTIBILITY BETWEEN THE GRAINS IN THE CANADIAN ADVANCED REGISTRY SWINE TESTING RATION[1]

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In a previous publication (3) reference was made to the Canadian Advanced Registry Swine Testing Ration. It consists of the three basal grains, barley, wheat and oats, with a protein-mineral supplement containing tankage, fishmeal, linseed oil meal, iodized salt and limestone. It was stated that a series of digestibility trials had been carried out to determine if any associative digestibility effects existed between the various components in the ration. The results of the first series of digestion trials were reported in that paper. Barley and tankage were fed together in varying proportions. It was concluded that there were no associative effects between these two feeds as far as total digestible nutrients were concerned. It was suggested that the high calcium in tankage rendered fats insoluble by forming calcium soaps.

In the present paper the grains, barley, wheat and oats, were fed in different proportions to one another with the protein-mineral supplement maintained at a constant level. The effect of the association of the grains with one another in different proportions on their digestibility was determined. The rations fed are given in Table 1. The numbers were assigned at random. These rations can be divided into four groups;

(1) The four components, barley, wheat, oats and the protein-mineral supplement fed alone as the sole ration.

(2) Barley and oats in varying proportions to one another with a constant quantity of protein supplement.

(3) Barley, wheat and oats in varying proportions to one another with a constant protein supplement.

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TABLE 1.—CONSTITUTION OF RATIONS

Ration No.	Proportions of grains in grain portion			Protein-mineral supplement added
	Barley %	Wheat %	Oats %	%
1	100	0	0	0
2	80	20	0	15
3	60	20	20	15
4	0	0	100	0
5	0	0	0	100
6	40	0	60	15
7	40	20	40	15
8	20	20	60	15
9	60	0	40	15
10	80	0	20	15
11	100	0	0	15
12	0	100	0	0

TABLE 2.—COEFFICIENTS OF DIGESTIBILITY OF NUTRIENTS OF TOTAL RATIONS

Ration* No.	Designation	Coefficients of digestibilities in per cent of following nutrients†						T.D.N. in dry matter %
		Dry matter	Organic matter	Nitrogen	Ether extract	Crude fibre	N.F.E.	
1	Individual values	82	83	85	38	22	89	82
		81	82	83	62	16	88	81
		80	82	83	38	21	88	80
		81	82	81	52	21	91	81
	Means	80.8	82.3	83.1	47.4	20.2	88.8	80.9
2	Individual values	82	84	83	87	27	89	83
		80	83	83	88	17	87	81
		78	81	82	83	12	86	79
		79	82	81	84	20	87	80
	Means	79.8	82.5	82.5	85.3	19.2	87.4	80.8
3	Individual values	78	81	83	87	20	86	80
		78	80	84	88	14	85	79
		76	79	82	83	16	84	78
		77	80	82	84	22	85	79
	Means	77.6	80.0	83.0	85.7	18.2	84.9	78.7
4	Individual values	64	66	84	82	10	73	67
		65	67	85	88	−1	74	68
		65	67	82	73	24	78	67
		63	65	84	83	8	71	66
	Means	64.3	66.1	83.6	81.4	10.2	74.0	67.2
5	Individual values	62	73	79	95	34	55	68
		63	75	79	98	35	18	70
		63	74	79	96	31	26	69
		61	72	79	92	22	52	67
	Means	62.2	73.8	79.1	95.4	30.5	37.8	68.3
6	Individual values	70	72	81	89	10	78	72
		72	74	83	91	8	80	74
		73	75	84	85	28	88	74
		68	70	80	87	6	76	70
	Means	70.6	72.9	82.0	87.9	13.1	80.6	72.5
7	Individual values	73	75	82	85	9	81	74
		76	79	85	88	14	84	78
		75	77	83	83	18	82	76
		74	76	83	84	10	82	75
	Means	74.4	76.6	83.0	85.0	12.8	82.0	75.7
8	Individual values	74	76	85	81	24	82	76
		74	76	84	85	13	82	76
		72	75	82	86	27	80	74
		70	72	81	88	11	78	72
	Means	72.7	75.1	82.7	85.0	18.6	80.2	74.6

(continued on page 478)

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* Rations corresponding to numbers listed in Table 1.

† Calculated to one place of decimals, shown here in units to comply with editorial policy.

TABLE 2.—COEFFICIENTS OF DIGESTIBILITY OF NUTRIENTS OF TOTAL RATIONS—*Concluded*

Ration* No.	Designation	Coefficients of digestibilities in per cent of following nutrients†						T.D.N. in dry matter %
		Dry matter	Organic matter	Nitrogen	Ether extract	Crude fibre	N.F.E.	
9	Individual values	73	76	82	87	5	81	75
		76	78	85	88	14	84	77
		72	74	76	79	15	80	73
		74	76	91	89	18	81	76
	Means	73.7	76.1	83.5	85.9	12.9	81.8	75.2
10	Individual values	78	81	85	90	21	85	79
		77	80	82	89	15	85	78
		76	79	81	76	21	82	77
		75	78	79	83	17	84	76
	Means	76.8	79.3	81.8	84.5	18.5	83.9	77.8
11	Individual values	80	83	84	87	22	87	81
		79	82	82	85	18	87	80
		75	78	78	80	11	84	75
		78	81	82	85	16	87	80
	Means	78.3	81.0	81.7	84.1	16.8	86.3	78.8
12	Individual values	89	90	91	79	18	93	90
		89	90	91	76	26	92	89
		88	89	91	69	21	92	88
		87	88	90	72	10	92	88
	Means	88.3	89.1	90.8	73.9	18.7	92.2	88.7

* Rations corresponding to numbers listed in Table 1.
† Calculated to one place of decimals, shown here in units to comply with editorial policy.

(4) Barley with the protein-mineral supplement and barley with wheat and the protein-mineral supplement.

The description of the feeds is as follows:

(1) *Barley*—"Velvet" variety, grown at the Experimental Farm, Ottawa, medium ground before each digestion trial.
(2) *Wheat*—No. 1 Western Canada Feed, medium ground before each trial.
(3) *Oats*—No. 1 Western Canada Feed, medium ground before each trial.
(4) *Tankage*—Canada Packers Company, Montreal, P.Q.
(5) *Linseed oil meal*—Sherwin-Williams Company Limited, Montreal. Screw-press process.
(6) *Fishmeal*—White fish meal, "Faster Fat" Company, Halifax, NS..

The chemical compositions of the feeds are given in Table 5 in the appendix. The animals received vitamin A and D feeding oil throughout the experiment. The amount of daily ration in each period was based on the live weights of the pigs. The ration was fed as a slop. The experimental animals were eight purebred Yorkshire barrows, numbered in the experiment 1 to 8. The live weights are given in Table 6 of the appendix.

TABLE 3.—STANDARD ERRORS OF MEAN COEFFICIENTS OF DIGESTIBILITY OF INDIVIDUAL NUTRIENTS. (MEAN OF FOUR VALUES)

Nutrient	Standard error
Dry matter	0.85
Organic matter	0.85
Nitrogen	1.38
Ether extract	1.79
Crude fibre	3.04
N-free extract	1.15
T.D.N. in dry matter	0.90

There were six digestion periods. Each period consisted of a pre-experimental sub-period of four days, a change-over sub-period of 3 days, a preliminary sub-period of 7 days and a collection sub-period of 10 days. The 12 rations were distributed to the 8 pigs through the 6 periods in a cyclic order. There were thus four replicated results for each ration. The experiment was commenced in May 1946 and concluded in September 1946.

PROGRESS OF THE EXPERIMENT

Animals 2 and 3 in period 1 and animal 4 in period 2 shed some hair in the feces. Animals 2 and 3 in period 3 and animal 6 in period 5 had some worms. Animal 2 in period 6 refused some feed during the second half of the collection sub-period. The results of the first five days were used in the calculations. Otherwise the experiment was completed satisfactorily.

RESULTS

A summary of the coefficients of digestibility is given in Table 2. In this table rations 1, 4, 5 and 12 consisted of the single components of the Advanced Registry ration. Rations 2, 3, 6, 7, 8, 9, 10 and 11 were mixtures of two or more of these components as shown in Table 1. To detect possible associative effects the following procedure was adopted. The results for rations 2, 3, 6, 7, 8, 9, 10 and 11 were arranged in columns of four replicates each and analysed statistically. The variance was separated into that due to rations and that due to replicates. This latter was taken as the error of the experiment. From it an estimate was made of the standard error of a single mean of four values. These standard errors for the different nutrients are given in Table 3. Using the values of the four components when each was fed alone, theoretical mean values of the mixed rations were calculated. These were compared with the observed means determined in the digestion trials. Using Fisher's method (1) for unique samples the differences between the observed and theoretical values were divided by the standard errors listed in Table 3. The results are given in Table 4.

It will be observed that for the dry matter, organic matter, nitrogen, nitrogen free extract and T.D.N. in dry matter, the differences were small and not statistically significant. With the ether extract there were larger differences, the observed values being higher than the theoretical. This

TABLE 4.—COMPARISON OF MEAN OBSERVED COEFFICIENTS OF DIGESTIBILITY OF TOTAL RATIONS WITH CALCULATED THEORETICAL VALUES. (MEANS OF FOUR OBSERVED VALUES, EACH RATION INCLUDES 15% OF PROTEIN-MINERAL SUPPLEMENT)

Designation	Mean coefficients of digestibility in per cent with difference and "t" values for following rations							
Ration No.	11	2	6	9	10	8	7	3
Barley %	100	80	40	60	80	20	40	60
Wheat %	0	20	0	0	0	20	20	20
Oats %	0	0	60	40	20	60	40	20
Dry matter								
Observed	78.3	79.8	70.6	73.7	76.8	72.7	74.4	77.6
Theoretical	78.2	79.4	69.6	72.5	75.3	70.9	73.8	76.6
Difference	+0.1	+0.4	+1.0	+1.2	+1.5	+1.8	+0.6	+1.0
"t"*	0.12	0.47	1.18	1.41	1.76	2.12	0.71	1.18
Organic matter								
Observed	81.0	82.5	72.9	76.1	79.3	75.1	76.6	80.0
Theoretical	81.3	82.5	72.7	75.6	78.4	73.9	76.8	79.6
Difference	−0.3	0.0	+0.2	+0.5	+0.9	+1.2	−0.2	+0.4
"t"*	0.35	—	0.24	0.59	1.06	1.41	0.24	0.47
Nitrogen								
Observed	81.7	82.5	82.0	83.5	81.8	82.7	83.0	83.0
Theoretical	81.6	82.7	81.7	81.7	81.7	82.8	82.8	82.7
Difference	+0.1	−0.2	+0.3	+1.8	+0.1	−0.1	+0.2	+0.3
"t"*	0.07	0.14	0.22	1.30	0.07	0.07	0.14	0.22
Ether extract								
Observed	84.1	85.3	87.9	85.9	84.5	85.0	85.0	85.7
Theoretical	74.5	72.2	80.6	77.5	73.7	82.8	79.8	76.4
Difference	+9.6	+13.1	+7.3	+8.4	+10.8	+2.2	+5.2	+9.3
"t"*	5.36	7.32	4.08	4.69	6.03	1.23	2.91	5.20
Crude fibre								
Observed	16.8	19.2	13.1	12.9	18.5	18.6	12.8	18.2
Theoretical	21.2	21.1	13.6	15.5	17.9	13.2	15.0	17.5
Difference	−4.4	−1.9	−0.5	−2.6	+0.6	+5.4	−2.2	+0.7
"t"*	1.45	0.63	0.16	0.86	0.20	1.78	0.72	0.23
N-free extract								
Observed	86.3	87.4	80.6	81.8	83.9	80.2	82.0	84.9
Theoretical	87.1	87.8	78.8	81.7	84.4	79.6	82.5	85.2
Difference	−0.8	−0.4	+1.8	+0.1	−0.5	+0.6	−0.5	−0.3
"t"*	0.70	0.35	1.57	0.09	0.43	0.52	0.43	0.26
T.D.N. in dry matter								
Observed	78.8	80.8	72.5	75.2	77.8	74.6	75.7	78.7
Theoretical	78.8	80.4	72.0	74.3	76.7	73.4	75.6	78.1
Difference	0.0	+0.4	+0.5	+0.9	+1.1	+1.2	+0.1	+0.6
"t"*	—	0.44	0.56	1.00	1.22	1.33	0.11	0.67

* "t" is determined by dividing the differences by the standard error in Table 3.
Nec. t at N of 24 for P of 0.05 = 2.03.
Nec. t at N of 24 for P of 0.01 = 2.72.

may possibly be due to an artefact. The amount of ether extract in the barley and wheat is quite low, being of the order of 1½ per cent of the dry matter. When these were fed as sole rations, a small amount of fecal fat of body origin would appreciably lower the apparent coefficients of digestibility. When mixed with the protein mineral supplement which contains considerable ether extract or with oats, also containing a fair amount of extract, the presence of the fecal metabolic fat would have less influence on the total apparent digestibility. This suggestion is borne out by the results of rations 6, 9 and 10 and also for 8, 7 and 3. As the relative quantities of barley or barley plus wheat were reduced, the differences between the observed and theoretical values became less.

With the crude fibre the differences were generally small. There were two rather wide variations. The experimental error was higher than for the other nutrients. The differences were not statistically significant. The digestibility was low.

On the basis of the contents of the T.D.N. in the dry matter and the digestibilities of the nitrogen, dry matter and organic matter, it was concluded that there were no associative effects between the three grains in the Advanced Registry Ration when they were fed with a constant quantity of the protein-mineral supplement.

SUMMARY AND CONCLUSION

1. A study was made of the associative digestibility between the three basal grains of the Canadian Advanced Registry Swine Testing Ration.

2. These grains were barley, wheat and oats.

3. Using eight pure bred Yorkshire barrows digestion trials were carried out on rations containing these three feeds in varying amounts.

4. A constant percentage of protein-mineral supplement was included in the rations.

5. It was concluded that there were no associative digestibility effects between the three grains.

ACKNOWLEDGMENTS

Acknowledgment is made to Messrs. Angus, Lalonde, Baker and Sample for assistance in carrying out the digestion trials, and to R. B. Carson and his staff for carrying out the chemical analyses of the samples.

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APPENDIX

TABLE 5.—CHEMICAL COMPOSITION OF FEEDSTUFFS (MEANS OF SIX VALUES)

Feedstuff	Original dry matter	Composition of dry matter				
		Ash	Protein*	Ether extract	Crude fibre	N-Free extract
Barley	87.12	3.00	13.86	1.63	6.34	75.17
Wheat	87.19	2.01	14.78	1.51	3.55	78.15
Oats	87.85	3.56	12.46	3.42	13.12	67.42
Tankage	90.61	27.78	55.55	8.71	2.43	5.53
Linseed oil meal	91.03	6.13	31.18	8.98	9.19	44.51
Fish meal	89.98	21.46	75.01	5.03	– –	– –

* Protein factors: Grains—N × 5.83 (Jones (2)) Linseed oil meal—N × 5.30 (Jones (2)) Tankage and fish meal—N × 6.25.

TABLE 6.—LIVE WEIGHTS OF ANIMALS IN KILOGRAMS*

Animal No.	Weights by following periods						Gains	
	1	2	3	4	5	6	Total	Daily #
1	68	87	106	117	132	143	75	0.54
2	73	90	109	121	134	149	76	0.55
3	64	79	98	103	120	136	72	0.52
4	67	83	101	119	131	146	79	0.57
5	63	80	100	114	131	146	83	0.60
6	63	80	99	111	115	133	70	0.50
7	60	77	93	107	127	148	88	0.63
8	73	81	101	117	132	148	75	0.54
Means	66	82	101	114	128	144	78	0.56

* Weights for each period are average of single weighings before and after collection periods.
# From middle date of two weighings in period 1 to middle date of two weighings in period 6 = 139 days.

A BACTERIAL SOFT ROT OF GLADIOLUS[1]

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[Received for publication July 28, 1950]

During the summer of 1949, attention was directed to a destructive soft rot of gladiolus plants in southwestern Ontario. As there appears to be no reference in the literature to a disease of this type, preliminary investigations were undertaken to determine its cause and to estimate its potentialities.

DISTRIBUTION OF THE DISEASE

The soft rot was first observed in a gladiolus plantation of four acres near Harrow, Ontario. The entire field had been irrigated a few times prior to the outbreak of the trouble, and at first it was thought that the occurrence of the disease might be connected with these applications of water. A survey of gladiolus plantings in the surrounding districts indicated, however, that the same disease was present in many unirrigated fields within a distance of 50 miles from Harrow, but to a lesser extent than in the first plantation. Late in the season, F. L. Drayton, of Ottawa, observed a disease with identical symptoms in a commercial gladiolus plantation at Oakville, Ontario, some 200 miles from Harrow, thus indicating that the disease was by no means localized in its occurrence.

REVIEW OF LITERATURE

In 1913, Severini (3), in Italy, described a soft rot of Gladiolus Colvillei and *Ixia maculata*, which, except for the symptoms on the corms, resembles the disease to be described in this paper. Severini isolated two apparently distinct bacteria. He found that one of these, which resembled *Bacillus aroideae* Towns., proved to be more virulent than the other, and demonstrated its pathogenicity on the species of *Gladiolus* and *Ixia* from which the organism was originally isolated and also on potato tubers. He named it *Bacillus ixiae.* Stevenson (4) reported the same disease in Holland.

According to Moore (1), a disease of gladiolus caused by *Bacillus omnivorus* van Hall (= *Bacterium carotovorum* (L. R. Jones) Lehm.), was described by Rainio (2) in Finland. It resembles in many respects the one found at Harrow and Oakville.

SYMPTOMS

The disease was most prevalent and severe on the gladiolus plants from which the upper portion of the flower-bearing stalks had been removed. On these plants, the symptoms consisted of chocolate brown, discoloured areas of variable extent, but usually beginning at the cut end of the flower stalk and extended downward towards the corm (Figure 1). Sometimes all of the leaves surrounding an infected flower stalk became involved, turned yellow, and died. In other plants, where the infection was confined to the remnant of the flower stalk or to the flower stalk and the immediately adjacent leaf sheath, various degrees of chlorosis and stunting were evident.

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[2] Officer-in-Charge.

Almost invariably, the pith of the brown flower stalk disappeared completely, with collapse and necrosis of the contiguous tissues. The basal portions of the inner leaves of affected plants were usually brown and necrotic.

The outer leaves were often water-soaked in appearance and deposits of a reddish gel-like exudate occurred between the broad leaf bases on the upper portion of the corms. Well defined oval to elongate lesions with a reddish brown halo were found on the leaves of the plant adjacent to the necrotic portions. In advanced stages, leaves were yellowed, streaked with brown, and finally became recumbent, dry, and chocolate brown.

An examination of the corms of affected plants throughout August showed that, in most of them, the disease had not extended into the corm itself, but in September, brown or black lesions, obviously the result of earlier stalk infection, were present on some of the corms. However, all of these lesions were superficial, and periodic examinations of these corms in common storage failed to detect any further progress of the disease. In late August, cormels were abundant on healthy plants, but there were few or none on the diseased ones (Figure 2).

Plants from small corms and some of those from which the flowers had not been cut, also exhibited the symptoms of the disease, as described above (Figure 2), in the absence of any visible injury to the flower stalks. In many such plants, however, corn borer larvae were present and apparently their tunnels served as infection courts for the pathogen.

THE CAUSAL ORGANISM AND ITS PATHOGENICITY

An examination of hand-sectioned gladiolus stalks and leaves from areas of incipient infection revealed an abundance of bacteria in the parenchymatous tissues. Isolations on potato dextrose agar from diseased tissues in all stages of the development of the disease invariably yielded bacterial colonies that were white and nearly spherical. The bacteria proved to be short rods, single or in chains, motile, and Gram-negative. The organisms isolated from the Harrow and the Oakville material were identical in every respect.

As the evidence provided by the symptoms, by the organism isolated, and by the microscopic appearance of the diseased tissue seemed to indicate that one of the soft-rotting organisms was involved, it was decided to carry out the pathogenicity trials on several of other plants and plant tissues in addition to the gladiolus.

Vigorously growing gladiolus plants in full bloom were selected for inoculation both in the greenhouse and in the field. Water suspensions of the bacteria from 24-hour old cultures on potato dextrose agar were swabbed on the exposed ends of freshly cut flower stalks with pieces of absorbent cotton and the exposed ends then bound with wax paper. Adequate control plants were prepared by swabbing the cut ends with sterilized water. The greenhouse plants were placed in a humidity chamber for 24 hours after inoculation and then returned to ordinary greenhouse conditions.

The other material used for inoculation included portions of potato tubers and carrot roots, and onion and tobacco plants. From the potatoes and carrots, cubes of internal tissues were removed aseptically and placed

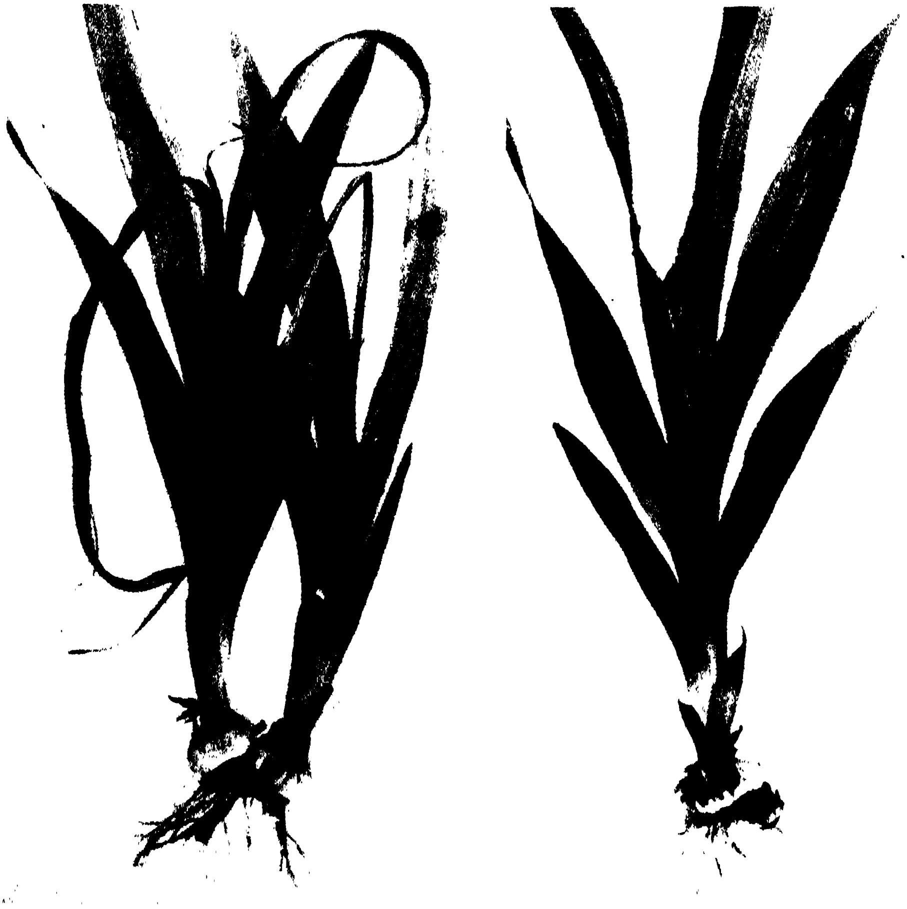

FIGURE 1. Gladiolus plants affected with bacterial soft rot at various stages after removal of flower stalks.

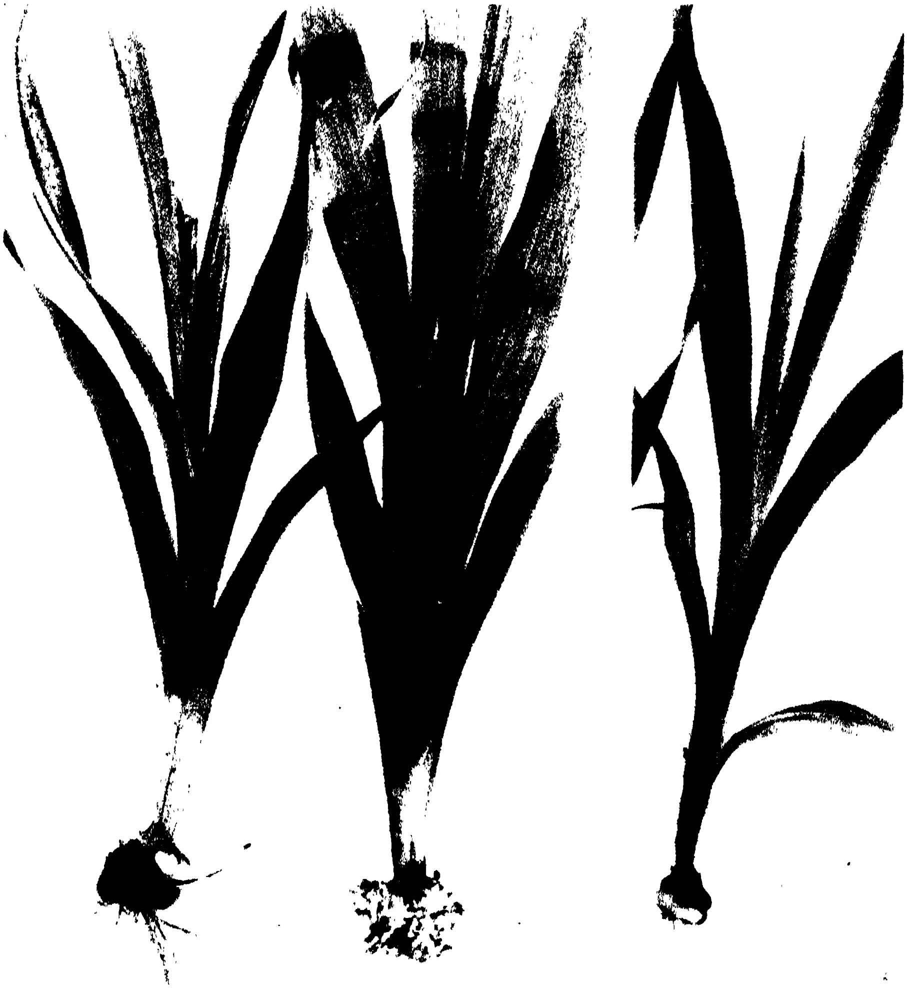

FIGURE 2. Left—Bacterial soft rot of gladiolus plant from which flower stalk was removed. Note absence of cormels. Centre—Healthy gladiolus plant at same stage. Right—Infection of young plant prior to flower formation.

in sterile Petri dishes containing moist filter paper. Some of the cubes of each kind were then inoculated with bacteria from the potato dextrose agar slants and incubated at 30° C. The tobacco plants used in the experiment were of the burley type and were approaching maturity in the field. The stalks were broken off and the bacteria applied in water suspensions to the exposed ends of the rooted portions. Sterilized water was applied to the control plants. The broken ends of all plants were then bound with wax paper to prevent these surfaces from drying out. For the onion plant trials, the Valencia variety, a Sweet Spanish onion, was chosen on account of its high susceptibility to common bacterial soft rot. Plants were carefully removed from the field and potted in the greenhouse. The inoculations were made by cutting off several of the central leaves and then applying water suspensions of the bacteria or water alone to the cut ends, which were then bound with wax paper.

TABLE 1.—RESULTS OF INOCULATING VARIOUS PLANT TISSUES WITH BACTERIA ISOLATED FROM DISEASED GLADIOLUS PLANTS

Host material	Number		Conditions of experiment	Results
	Inoc.	Check		
Potato tuber (cubes)	4	4	Held at 30° C.	After 5-7 days*, all inoculated cubes showed partial to complete rotting. Checks healthy.
Potato tuber (cubes)	4	4	Held at 30° C.	(ditto)
Carrot (cubes)	4	4	Held at 30° C.	After 5 days, all inoculated cubes completely rotted. Checks healthy.
Carrot (cubes)	4	4	Held at 30° C.	(ditto)
Onion plants	6	6	Greenhouse temperature ± 30° C.	After 7 days, all inoculated plants showed partial rotting of central region of bulb. After 14 days, 3 bulbs completely rotted. Checks healthy.
Onion plants	5	5	Greenhouse temperature ± 26° C.	After 14 days, all inoculated bulbs partially or completely rotted.
Tobacco plants (stalks)	6	6	Field temperature	Negative. No signs of rotting.
Gladiolus plants	10	10	In field. Watered regularly.	After 5 days, inoculated flower stalks showed variable amount of necrosis in region of pith and watersoaking of external tissues. After 10 days, necrotic portion in most cases extended to corm. After 2 weeks, inoculated plants manifested typical field symptoms.
Gladiolus plants	5	5	Greenhouse temperature ± 30° C.	(ditto)

* All inoculated potato tuber, carrot and onion tissue manifested the disagreeable odour characteristic of tissues affected with bacterial soft rot, caused by *Erwinia carotovora*.

The results of these pathogenicity trials are summarized in Table 1. From these it is evident that all of the potato tuber and carrot cubes, the onion bulbs, and the gladiolus stalks rotted within a period of 5-14 days after inoculation with pure cultures of the bacteria isolated from the diseased gladiolus plants. On the other hand, the tobacco stalks remained unaffected, despite the fact that tobacco plants are known to be subject to bacterial soft rot.

The development of the disease on the inoculated gladiolus plants proved to be highly consistent. Ten days after inoculation, all of the plants showed discoloration of some or all of the leaves, extending down to the corms. This condition was accompanied by a softening and necrosis of the flower stalks and the adjacent tissues. After twenty days, all of the inoculated plants were stunted and developed smaller corms than did the uninoculated plants. The inoculated plants developed few or no cormels, and without exception, they showed varying degrees of chlorosis, accompanied by necrotic lesions near the base of the stalks. At that stage, the latter were in most cases brown and collapsed. Re-isolations from the diseased tissue of the inoculated plants yielded, without exception, bacteria which were similar in every respect to the ones isolated from the naturally infected plants.

It should be emphasized that the outstanding symptoms of this gladiolus disease are the chocolate brown to black discoloration of the flower stalks and the adjacent leaf sheath tissue and the disappearance of the pith.

CONCLUSIONS

It would appear that the organism responsible for this gladiolus disease closely resembles *Erwinia carotovora* (L. R. Jones), Holland. The symptomatology of the disease in naturally infected and artificially inoculated plants closely resembles that of the bacterial soft rot ascribed to *E. carotovora* in several vegetables and other plants. In addition, the pathological histology of the affected tissues, the range of susceptible plants, and the cultural characters and morphology of the causal organism, all provide evidence for this tentative identification.

It is possible that the environmental conditions prevailing in southern Ontario during 1949 were particularly favourable to the development of a disease of this kind. On the other hand, if these conditions provide the explanation for the sudden occurrence of the disease and the consequent damage to the gladiolus plants, it seems strange that an organism so common and widespread in the district has not caused the disease previously. The circumstances surrounding its possible recurrence in the immediate future may provide the explanation and indicate whether or not special measures for its control should be undertaken.

ACKNOWLEDGMENT

Acknowledgment is due W. G. Benedict of the Harrow laboratory for assistance rendered throughout the period of this investigation.

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BOOK REVIEW

BOTANY by W. W. Robbins and T. E. Weier. John Wiley & Sons Inc., New York. 480 pages. 1950. $5.00.

Among the recently published texts in botany is this attractively illustrated book, written by two members of the staff of the University of California. The authors have attempted to arouse the interest of the student by giving an appreciation of the scope of botany and of its relation to agriculture, forestry, and medicine. The double-column format, together with nearly 500 well-labelled illustrations, combine to produce an interesting and easily read book.

The arrangement of the subject matter is much the same as is found in most general botany texts. The various parts of the plant (stem, root, etc.) are dealt with first, and then follows a discussion of the different groups of plants (algae, fungi, etc.). Among the topics covered in the 22 chapters are: The plant world; classification and naming of plants; plant cell; stem; roots; leaf; flower; fruit, seed, and seedlings; inheritance; plant as a living mechanism; groups of plants; fission plants; algae; fungi; viruses; bryophytes; vascular plants; and evolution.

Two of the errors in the book are of especial concern to Canadians. It is annoying, after reading on page 27 that "the coniferous forests of North America are shown in Fig. 2.10", to find that Fig. 2.10 features only the United States, with but a blank space to the north of the U.S.-Canada border. The second error is on page 227, where reference is made to *13* Canadian provinces. Fortunately the botanical information in the text is more reliable than the above-mentioned references and as an introduction to plant science this book is a valuable addition to the field.

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FURTHER TESTS OF INSECTICIDES AGAINST BLACK FLIES (DIPTERA: SIMULIIDAE) AND A CONTROL PROCEDURE[1]

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[Received for publication July 27, 1950]

INTRODUCTION

Preliminary investigations into the possibility of controlling black flies in the larval stage with some of the synthetic organic insecticides were made at Churchill, Manitoba, in June and July, 1947, and have already been reported (5). This work was continued at Churchill and in the vicinity of Whitehorse, Y.T., in 1948 and 1949, with special attention to the possibility of obtaining a satisfactory mortality of the eggs and pupae, and of arriving at a practicable procedure foi routine control measures.

During this time, similar work on Alaskan species has been reported. Gjullin and his co-workers (3, 4) obtained control of the larvae of *Simulium venustum* Say, *S. vittatum* Zett., and *Eusimulium latipes* (Mgn.) with dosages of various DDT formulations from 4.5 to 10.5 p.p.m.-minutes, and reported that *Prosimulium hirtipes* Fries could be detached by adosage of 1.8 p.p.m.-minutes, which closely approaches that found satisfactory against *S. venustum* at Churchill in 1947. The same workers obtained satisfactory results from aerial applications. Using methoxychlor and dichlorodiphenyl dichloroethane, Travis (11) has reported results comparable to those obtained with DDT; he also reported parathion more effective than other materials, but not sufficiently so to warrant its use. Travis considered DDT in fuel oil solution the most satisfactoiy formulation, applied for an unspecified time at 0.4 p.p.m., and recommended aerial spraying as a method of application. The species referred to in his paper are *S. venustum*, *S. vittatum*, and *Prosimulium fulvum* Coq. Austialian workers (8) have reported methods for the laboratory rearing of black flies, and have shown interest in aerial applications of DDT. Work is planned on the control of *Simulium neavei* Roub. over a large area in East Africa, in connection with onchocerciasis (2). In Canada, *Simulium arcticum* Mall. has been controlled, for a proved distance of 17 miles and a possible distance of 90 miles downstream, by a single aerial application of DDT in fuel oil at 4.68 p.p.m.-minutes, but 2.38 p.p.m.-minutes failed to give control (1).

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[1] The results herein reported are from a research project which forms part of a program of studies on the biology and control of biting flies in northern Canada being carried out by the Division of Entomology, Science Service, Canada Department of Agriculture, on behalf of the Defence Research Board, and with the co-operation of that organization and other agencies.

[2] Defence Research Board. Assistant Professor of Entomology, University of Alberta, Edmonton, Alberta.

MATERIALS TESTED

Tests were made against one or more of the aquatic stages of black flies, and against other ecologically related organisms in the same environment, with each of the following materials:—

DDT (*Technical Grade*):

10 per cent W/V solution in fuel oil plus Velsicol A.R. 50 as auxiliary solvent.

25 per cent emulsion concentrate also containing 65 per cent of xylene and 10 per cent of Triton X-100.

Gamma Benzene Hexachloride:

An emulsion concentrate supplied by Canadian Industries Limited containing 4 per cent of gamma isomer.

A wettable powder containing 25 per cent of gamma isomer.

An oil solution containing 1.3 per cent W/V gamma isomer.

Parathion:

A wettable powder containing 25 per cent of active material.

A wettable powder containing 15 per cent of active material.

An emulsifiable concentrate containing 20 per cent of active material.

Methoxychlor:

A 50 per cent wettable powder prepared from a technical material containing 44 per cent of methoxychlor and 6 per cent of other isomers and reaction products.

Aldrin (*Julius Hyman's Compound 118*):

A wettable powder containing 25 per cent of active materials.

An emulsion concentrate containing 45 per cent of active materials.

10 per cent W/V solution in fuel oil with auxiliary solvent.

All of these were prepared from the technical compound containing 75 per cent of the active principle itself, and 25 per cent of related active compounds.

Dieldrin (*Julius Hyman's Compound 497*):

A wettable powder containing 15 per cent of active materials.

An emulsion concentrate containing 25 per cent of active materials.

5 per cent W/V solution in fuel oil with auxiliary solvent.

All of these were prepared from the technical compound containing 60 per cent of the active principle and 40 per cent of related compounds.

1, 2, 4-trichlorobenzene:

An emulsion concentrate containing 90 per cent of active material and 10 per cent of Triton X-100.

Except for one experiment, all exposure or application times were 15 minutes, so that the concentration in parts per million is obtained by dividing the dosage figures given in p.p.m.-minutes in the following tables by 15. Calculation of concentration was based on the percentage of the technical material in the formulation employed, except that for benzene hexachloride it was based on the percentage of gamma isomer.

The trichlorobenzene preparation was tested in 1949, in the hope that it might throw some light on anomalous results previously obtained with benzene hexachloride. 1, 2, 4-trichlorobenzene is an important breakdown product of benzene hexachloride (6).

Some difficulty was experienced in obtaining satisfactory emulsions and suspensions with the formulations of dieldrin, a stubbornly hydrofuge material. With the possible exception of methoxychlor, none of the wettable powders suspended well enough in the stream water in which they were used to permit their application with the dispenser described in the appendix to this paper.

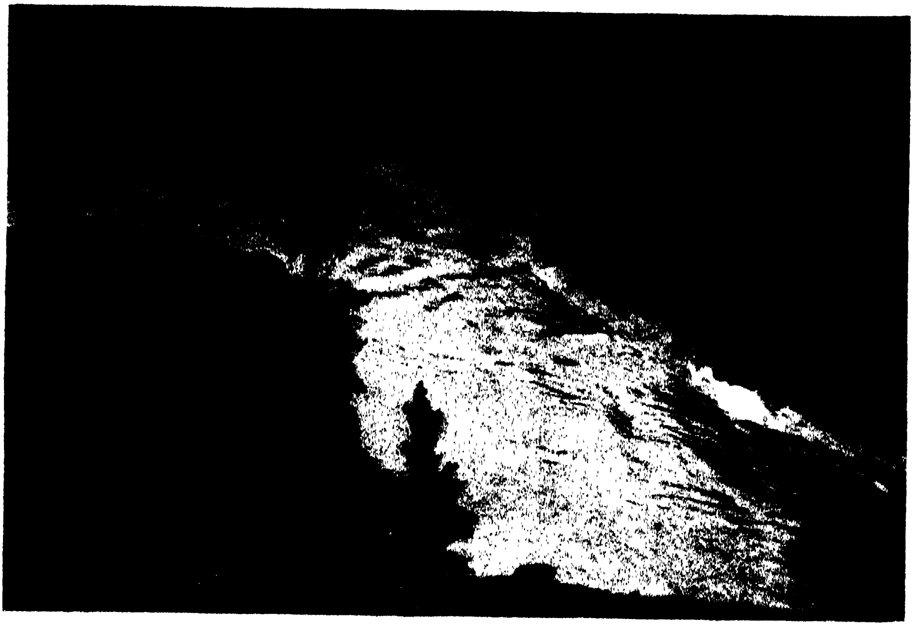

FIGURE 1. Miles Canyon, 6 miles south of Whitehorse, Y.T., on the Lewes River, treated with DDT in 1948. Photographed in 1949.

FIGURE 2. Prototype dispenser applying aldrin emulsion in experiment 6.

FIGURE 3. Modified dispenser applying DDT in fuel oil from a road culvert. Experiment 27.

FIGURE 4. The treated stretch of Stony Creek, experiment 8.

OVICIDE EXPERIMENTS

Tests of the toxicity of these insecticides to black fly eggs were severely limited by the difficulty of obtaining eggs in suitable quantity and situation. When egg masses were found they were usually located at the upper end of a stretch of stream suitable for black fly breeding, so that it was not possible to divide the stream into a stretch for treatment and an upstream stretch for a check. Eggs located in this situation at Churchill in 1948, in all probability those of *Simulium venustum*, were taken into the laboratory for small-scale tests.

All the eggs used in each test were from a single egg mass, and probably of comparable age. Parathion suspensions and benzene hexachloride emulsions were tested on these eggs. Batches, consisting of several hundred eggs on the vegetation on which they were laid, were immersed for 15 minutes in dilutions of the insecticides prepared with stream water, and stirred gently during the tests. The eggs were then rinsed off with plain stream water and placed in tubes plugged with wet cotton. Check batches were similarly treated in plain stream water. Counts of the percentage hatch were made when hatching was complete in the check batches, and were confirmed 3 days later. The results are given in Table 1.

TABLE 1.—TOXICITY OF GAMMA BHC AND PARATHION TO BLACK FLY EGGS

Insecticide	Concentration p.p.m.	Dosage p.p.m.-min.	Percentage hatch
Parathion	0.2	3.0	80
	0.5	7.5	2
	1.0	15.0	0.3
Check 1 (untreated)	—	—	88
Gamma BHC	0.2	3.0	83
	0.5	7.5	15
	1.0	15.0	4
Check 2 (untreated)	—	—	99.5

No egg masses were found at Whitehorse in 1949, so that no further ovicidal data could be obtained. This may have been because it was too early in the season, or possibly because, as Smart (9) has suggested for *Prosimulium hirtipes*, the commoner species there may lay their eggs singly.

LARVICIDE EXPERIMENTS

The methods of test employed were virtually the same as those already described (5). Two additional methods of measuring the flow of streams were used, a Gurley flow meter, and a pitot tube with S.A.E. 10 oil as the manometric fluid. The float method which was used in 1947 was checked against the Gurley flow meter, and gave figures which agreed within 5 per cent. The pitot tube was also calibrated against the flow meter, and gave less consistent results; this equipment is cheaper and less cumbersome than the flow meter, however, and as the method is quicker it may prove suitable for use in routine control.

TABLE 2.—SUMMARY OF DATA FROM TREATMENT OF STREAMS NEAR CHURCHILL, MANITOBA, WITH PARATHION AND DDT AGAINST LARVAE OF BLACK FLIES, 1948

Experiment no. and date of treatment	Observed treated length of stream in yards	Average flow in cusecs	Temperature, °F.	pH	Insecticide formulation	Method of application	Dosage p.p.m./min.	Larval population		% emergence from pupae	
								Before treatment	24 hr. after treatment	Before treatment	After treatment
1. July 7	200	0.62	62	7.5	15% Parathion wettable powder	Medicine dropper	3	Moderate	Nil	67	22
2. July 22	300	0.33	64	8.0	15% Parathion wettable powder	Graduate and paddle	0.5	Light	Light	67	73
3. July 22	1400	30.8	62	7.5	10% DDT in fuel oil	Graduate	1.7	Moderate	Nil	—	—
4. July 24	5300	141	66.5	7.5	10% DDT in fuel oil	Graduate	1.04	Light	Nil	—	—

Experiments in 1948

Conditions at Churchill in 1948 were drier than in 1947, and this, together with the earlier use of considerable areas of country for experimental spraying with DDT from the air, prevented extensive work with insecticides in streams. The results obtained with DDT in 1947 were, however, confirmed by applications to Eastern Creek, on the same stretch as that employed in 1947; and to Herriot Creek, a well defined channel with substantial flow, entering the Churchill River from the west, some 25 miles south of Churchill. A length of nearly 3 miles of Herriot Creek was under observation.

Two preliminary tests of a 15 per cent parathion wettable powder were made, one in a small stream draining into a lake on the tundra east of Churchill camp, and one in Middle Eastern Creek. The results obtained in these four tests are given in Table 2.

At Whitehorse, in July, 1948, van Steenburgh and Twinn organized and made observations on a practical application of DDT in oil solution in the Lewes River. In an unpublished report, Twinn has stated as follows:

> "The Lewes River was examined at several points from Marsh Lake northward to Whitehorse, a distance of about 20 miles. Throughout this length it flows swiftly and was found to be supporting an enormous population of black fly larvae and pupae, the larvae apparently predominating. Pupae taken from aquatic grasses on the rocks, in the shallow margin of the tumultuous rapids in the canyon south of Whitehorse, were *Simulium arcticum* Mall. Pupae of this species and *S. venustum* Say, and ones with 4 and 8 filaments in the respiratory tufts, probably *S. pugetense* D. & S. (=*Eusimulium costatum* Fried.) and *S. ottawaense* Twinn (=*S. decorum* Wlk.), were found in the river at points south of the canyon.......
>
> "The site chosen for applying the insecticide was a weir or dam north of Marsh Lake, about 20 miles south of Whitehorse .... The drums of 5% DDT (in kerosene) were ... rolled on to the dam and spaced at equal distances across the river. Rough measurements at this point indicated the river to be 375 ft. wide, 10 ft. deep and flowing at 5+ ft. per second .... The calculated flow was 17,625 cu. ft. per second ... The temperature of the water was 62°F.
>
> "When all was ready, on a signal the small bungs were removed simultaneously from the five drums, and the large bungs loosened. The DDT-oil solution poured fairly evenly into the river and all drums emptied themselves in 10 minutes, giving an actual dosage of 1 : 7, 135,000 at the point of application for that period (1.4 p.p.m.-minutes).
>
> "The next morning, fourteen hours later, ... several known infested areas were reexamined along the margins of the rapids in the canyon about 15 miles below the point of application and also in Whitehorse about 20 miles therefrom. All larvae had disappeared from grasses and vegetation in the swift water, and only the pupae remained."

This was the largest river that had been successfully treated by ground application in Canada up to that time. The canyon referred to in this account is illustrated in Figure 1.

Experiments at Whitehorse in 1949

Most of this work was done in the area between Carcross and Whitehorse, Y.T., where a great number of streams in which black flies breed are readily accessible for survey and treatment, from the North West Highway System. Little knowledge of the fauna of these streams existed, and it was found during these experiments that the populations of species important as biters of man were relatively small; several species new to science were found. The work was done rather early in the season, which was very dry, the flow in the Lewes River in July being only about one-tenth of that in 1948. This lack of knowledge concerning some of the species involved is probably of little significance in relation to larval control, since no specific differences in susceptibility to insecticides have yet shown up in this work.

TABLE 3.—SUMMARY OF DATA FROM TREATMENT OF STREAMS NEAR WHITEHORSE,

Expt. no.	Date treated	Observed treated length in yards	Average flow in cusecs	Nature of bottom	Temp. °F.	Insecticide formulation
1	June 25	210	9.1	Fine gravel	40	497 emulsion concentrate
2	June 25	120	1.0	Gravel	42	497 emulsion concentrate
3	June 15	1000	1.4	Mud and sand	56	497 wettable powder
4	June 15	1500	1.7	Sand	40	497 wettable powder
5	June 29	250	2.5	Mud and rocks	45	497 in fuel oil
6	June 15	250	32.7	Rock and boulders	50	118 emulsion concentrate
7	June 15	150	0.58	Gravel	36	118 emulsion concentrage
8	June 15	800	14.7	Gravel and boulders	44	118 wettable powder
9	June 25	150	0.32	Sand and gravel	46	118 wettable powder
10	June 27	2000	6.6	Mud and sand	40	118 in fuel oil
11	June 15	100	1.0	Mud	42	Methoxychlor wettable powder
12	June 15	350	41.7	Mud and sand	54	Methoxychlor wettable powder
13	June 15	100	2.2	Gravel	38	Trichlorobenzene
14	June 15	100	0.39	Gravel and stones	38	Trichlorobenzene
15	June 25	120	1.3	Mud and sand	54	Trichlorobenzene
16	June 18	800	23.2	Mud and sand	53	gamma BHC emulsion
17	June 18	150	2.3	Mud and sand	41	gamma BHC emulsion
18	June 27	180	10.5	Sand	42	BHC wettable powder
19	June 23	50	7.5	Sand and gravel	38	BHC wettable powder
20	June 25	150	2.9	Sand	40	BHC in fuel oil
21	June 25	190	3.2	Gravel and boulders	40	BHC in fuel oil
22	June 23	200	1.8	Gravel	38	Parathion emulsion concentrate
23	June 23	150	1.4	Gravel and boulders	44	Parathion emulsion concentrate
24	June 25	320	0.87	Boulders	43	Parathion wettable powder
25	June 25	450	6.1	Sand and gravel	50	Parathion wettable powder
26	June 25	100	3.1	Gravel	42	10% DDT in fuel oil
27	June 25	250	4.6	Gravel	41	10% DDT in fuel oil
28	June 25	330	168	Rock and boulders	48	10% DDT in fuel oil

Y.T., WITH VARIOUS INSECTICIDES AGAINST LARVAE OF BLACK FLIES, 1949

Method of application	Dosage in p.p.m./min.	Larval control	Pupal control	Species present
Graduate	3	Excellent	—	*P. hirtipes*
Graduate	7.5	Good		
Graduate	3	Slight		
Graduate	7.5	Moderate	Doubtful	*Prosimulium* "J" and *P. hirtipes*
Eye dropper	15	Good		*S. arcticum*
Dispenser	3	Slight		
Dispenser	7.5	Slight		
Graduate	3	Slight	Moderate	*Prosimulium* "J"
Graduate	7.3	Moderate	Nil	*P. onychodactylum* D. & S. *Cnephia* "G" *P decemarticulatum* Twinn
Eye dropper	15	Slight		*P. hirtipes* and *E. costatum*
Graduate	3	Nil		
Graduate	7.5	Slight	Nil	*E. costatum*
Graduate	4.1	Nil		
Graduate	10.8	Nil		
Graduate	30	Nil	Good	*S. perissum* and *E. latipes*
Dispenser	7.5	Slight	Slight	*Cnephia* "D", *S. arcticum*
Dispenser	15	Slight	Nil	*E. costatum* and *P. hirtipes*
Graduate	3	Good		
Graduate	7.5	Good	Slight	*P. hirtipes* and *E. costatum*
Eye dropper	1.5	Good	*	*P. hirtipes*
Eye dropper	3	Good		
Graduate	1.5	Slight	—	*P. hirtipes*
Graduate	7.5	Moderate	Good	*P. hirtipes* and *E. costatum*
Graduate	7.5	Slight	—	*P. hirtipes*
Graduate	15	Excellent	Moderate	*P. hirtipes*
Dispenser	1.5	Excellent	Nil	*P. hirtipes* and *E. costatum*
Dispenser	1.5	Good		
Dispenser	1.5	Not assessed		*S. arcticum*

* % emergence greater in the treated than in the check batch; see explanation in section on toxicity to pupae.

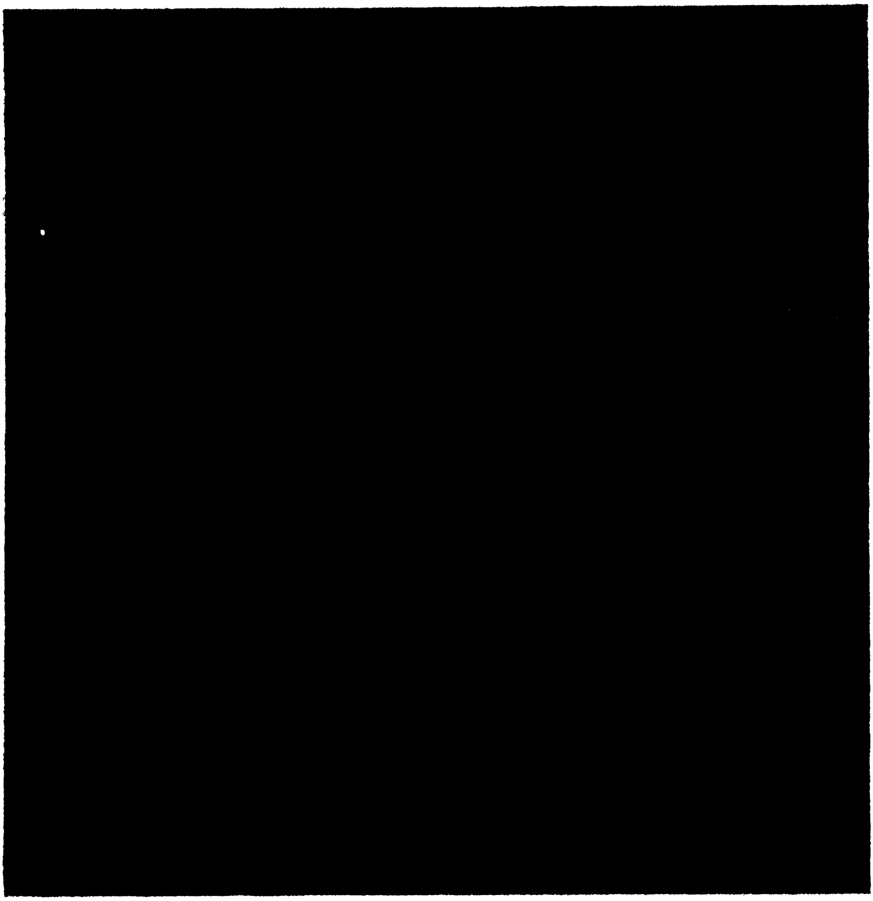

FIGURE 5. Close-up of modified dispenser applying DDT in fuel oil in experiment 27.

A new method of application was used in many of these tests, involving gravity flow from a tank fitted with a globe valve and a manometer connected across an orifice plate. Several changes were made in this apparatus during the course of the experiments, some of which are illustrated in Figures 2 and 4. In the appendix the apparatus is described in its final form, together with simplified instructions for its use in routine control measures.

The results obtained in these experiments are summarized in Table 3. Observations were made on the pH and salinity of all streams, and on the oxygen content of the water from the first 14 streams treated. Oxygen determinations were made by Winkler's method, and were discontinued when it was found that nearly all readings fell between 89 and 94 per cent of saturation. Readings were taken, however, for any stream which appeared exceptional, and in experiment 15, 100 per cent saturation was recorded, whereas that in experiment 20 was only 61 per cent. With the exception of experiment 2 (7.85) and experiment 5 (7.5), all streams had pH values between 8.15 and 8.75, measured on a Beckman pH meter. In all streams the salinity of the water, estimated by a nephelometric method, was less than 1 part of chlorides per million.

Specific identifications were made by G.E. Shewell of Systematic Entomology, Division of Entomology, Ottawa, for the most part from rather fragmentary material of samples taken to assess toxicity to pupae. Where no pupal sample was secured, specific identification has not, as yet, been possible, but there is good reason to suppose that the two commoner species, *Eusimulium costatum* Fried. and *Prosimulium hirtipes* Fries, were present in most of the smaller streams, and *Simulium arcticum* Mall. in the rivers. The occasional discovery of mature larvae with 6 filaments in the histoblasts of the pupal respiratory structures, together with earlier observations on the fauna in this area, suggest that larvae of the important biting species *Simulium venustum* Say may have composed a significant proportion of the populations actually killed by the treatments. Although some emergence of adults of *P. hirtipes* had already taken place when this work was started, this species did not live up to its reputation, and no observations were made of its biting man. Shewell's notes on the other species are given below with the accounts of the streams in which they were found.

Eight of the larger streams treated were identified by map names: Leguiel Creek (expt. 5), Stony Creek (expt. 8), Porter Creek (expt. 10), Little River (expt. 12), Judas Creek (expt. 16), McIntyre Creek (expt. 18), Robinson Creek (expt. 25), and Watson River (expt. 28). The remaining smaller streams were identified by milepost numbers of their crossings of the Alaska Highway, or of side roads. In addition to the 28 streams that were treated, 20 streams were surveyed and showed promise of being suitable for experimental treatment later in the season. Some typical streams are illustrated in Figures 2, 3, 5, and 6.

The following streams showed features of special interest:

Stony Creek (*Expt. 8*) *and Stream No. 4.*—Both of these streams flow into the Takhini River to the northwest of Whitehorse, the former on the left bank, about 30 miles above its efflux into the Lewes River (Figure 5), and the latter on the right bank about 20 miles above the efflux, crossing the Alaska Highway at mile 939.2. These were the only streams from which the species *Prosimulium* "J", apparently an undescribed species, was taken. They differ markedly in character: Stony Creek being swift, clean, and appropriately named; No. 4, sandy, sluggish, and cloudy.

Experiment No. 9.—This little stream was the only source of specimens of *P. onychodactylum* D. & S. and of a new species, *Cnephia* "G". The pupal filaments of the latter resemble the antlers of caribou. The stream crosses the Carcross Road at Milepost 16, draining from a swamp just to the east of it, and pursues a somewhat vague course through dense vegetation and deadfall.

Experiment No. 15.—Two species, *S. perissum* D. & S. and *Eusimulium latipes* (Mgn.), were recorded from this stream and nowhere else in the locality. The former was more abundant than any other species in any other stream; the latter was represented by a single empty pupal skin. The physical characteristics of the stream were also unusual. It is a small, smooth, slow-flowing, warm stream, winding across an old lake-bed. It was one of the few streams with much aquatic vegetation, and the only one in which the oxygen content of the water reached saturation. The stream crosses the Carcross-Tagish highway 9.5 miles from its junction with the Alaska Highway.

Judas Creek (*Expt. 16*).—*Cnephia* "D", a species closely related to *C. boreale* (Mall.), was taken from this stream only; it was common there in association with *Simulium arcticum*. The larvae in this stream reacted to the benzene hexachloride emulsion in a characteristic manner, many releasing on threads before the end of the treatment, but control was incomplete and reinfestation rapid. Judas Creek crosses the Alaska Highway at mile 872.1.

Experiment No. 20.—*P. hirtipes* was found in this stream, which crosses the Carcross-Tagish highway 22.2 miles from its junction with the Alaska Highway.

Watson River (*Expt. 28*).—By treating this river with DDT at a point west of Robinson, on the Carcross highway, and making an assessment at its efflux into Lake Bennett, it was hoped to secure observations on the effect 20 miles below the application point. The river, however, proved inaccessible at the latter point.

When no living larvae could be discovered in the observed length of stream after treatment, this was recorded as excellent control; control was recorded as "nil" if no change in the population could be detected. If scattered larvae were found throughout the observed length, or if the distance for which the larvae were eliminated or killed was less than the observed length, control was classed as slight, moderate, or good, according to the degree of infestation remaining in comparison with the initial population. Treatments were assessed as nearly as possible 24 hours, and always between 18 and 30 hours, after application.

TOXICITY TO PUPAE

For insecticides applied to streams the only simple method for estimating the toxicity to the pupal stages of black flies is to take samples of pupae before and after treatment, and record the percentage of adult emergence from each sample in the laboratory. The second pupal sample was usually taken 24 hours after treatment, since the stream was revisited for assessing larval mortality after this interval and since it was desirable to allow adequate time for the "tail" of insecticide to pass. This method is reasonably satisfactory with species such as *Simulium venustum* and *S. perissum*, which give a high and predictable emergence under laboratory conditions; but it was not satisfactory with species such as *Prosimulium hirtipes*, which did not emerge in tubes plugged with wet cotton.

For this reason, the more valuable results on this question have been obtained at Churchill, where *S. venustum* is the dominant species. Results obtained in 1948 in conjunction with tests against larvae are recorded in Table 2, and other data obtained at Churchill in 1948 and 1949 are summarized in Table 4. Such limited data as were obtained at Whitehorse in 1949 are given in Table 3; a blank space in the pupal control column indicates that no pupal samples were obtained; a dash indicates that samples were secured but that no emergence took place from either treated or untreated pupae.

Observations during the course of this work have shown clearly that the resistance of black fly pupae to most insecticides increases sharply with age. The same appears to apply to the resistance to exposure to the air,

FIGURE 6. Part of the stream treated in experiment 6, downstream from that in Figure 2.

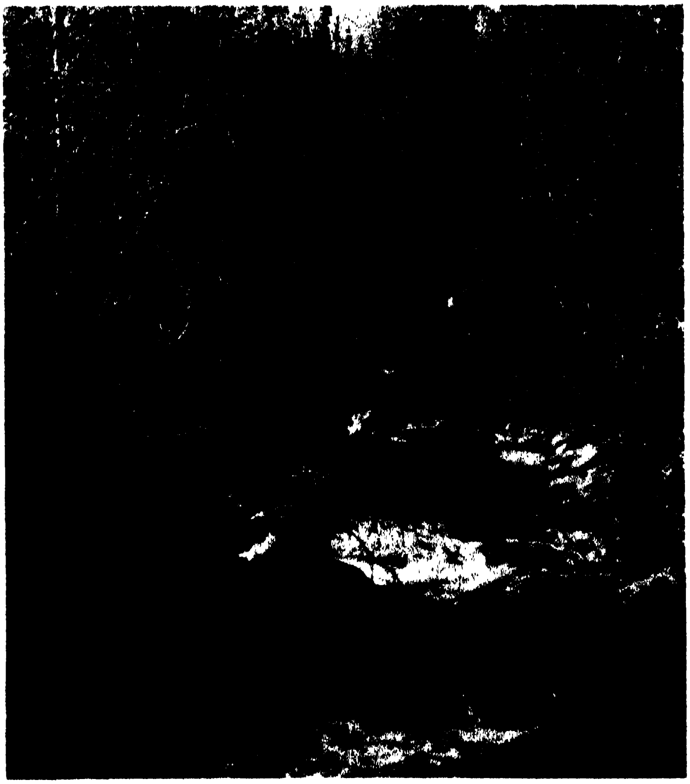

FIGURE 7. The stream treated in experiment 13.

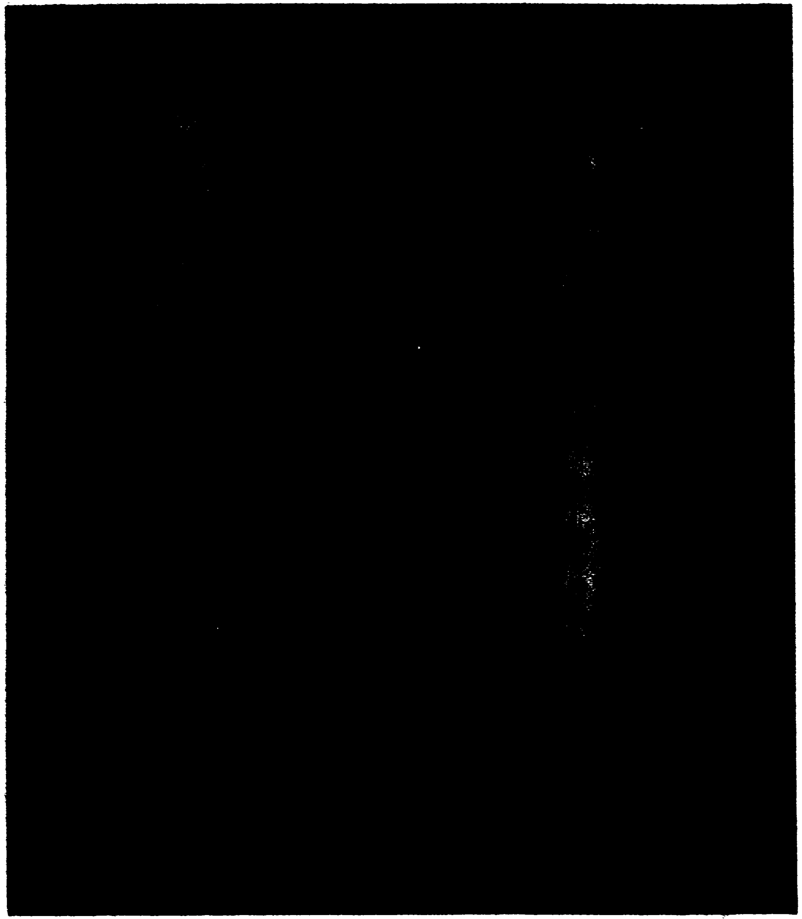

FIGURE 10. Typical black fly larvae (after Twinn).

FIGURE 11. Typical black fly pupae (after Twinn).

TABLE 4.—SUMMARY OF DATA ON TOXICITY OF BENZENE HEXACHLORIDE AND TRICHLOROBENZENE TO PUPAE OF *Simulium venustum*, CHURCHILL, MANITOBA, 1948 AND 1949

Date	Insecticide formulation	Dosage p.p.m./min.	No. of pupae	% Emergence	
				Before treatment	After treatment
Aug. 5, 1948	BHC oil solution	1.8	2× 100	26	44
Aug. 5, 1948	BHC oil solution	3.6	2 × 100	42	48
Aug. 5, 1948	BHC oil solution	3.6	2 × 100	29	80
Aug. 5, 1948	BHC oil solution	7.2	2 × 100	9	80
Aug. 4, 1949	1, 2, 4-trichlorobenzene	15	2 × 50	78	84
Aug. 4, 1949	1, 2, 4-trichlorobenzene	30	2 × 50	78	88
Aug. 11, 1949	1, 2, 4-trichlorobenzene	60*	2 × 25	92	68

* Test conducted in water from the stream.

the greater the average age of a sample of pupae, the greater the percentage emergence of adults in tubes plugged with wet cotton. A random sample of pupae taken 24 hours after the successful application of a larvicide is of greater average age than a check sample taken before treatment or from an untreated part of the stream. The amount of this difference in average age depends on the frequency distribution of age groups; at the commencement of pupation it approaches its maximum possible value of 24 hours, and may have a significant effect on the percentage emergence. In spite of this effect, however, there is evidence that treatment with benzene hexachloride in oil solution has a stimulating effect on the emergence of the adult, although benzene hexachloride emulsions (5) and suspension effect a measure of control.

EFFECTS ON OTHER ORGANISMS

The influence of insecticidal treatments of streams on other organisms may be of considerable importance in any one of three main ways. Organisms utilized as food by black fly larvae may be affected. Apart from a brief reference (7) to the difficulty of sectioning these larvae on account of the siliceous skeletons of diatoms in the alimentary canal, and an account by Smart (10) indicating how catholic their tastes may be, published information on the nature of these organisms is scarce. Of greater importance is the possible effect on the predators of black fly larvae, either directly or through other organisms used as food. Although many insects, in particular predatory larvae of Trichoptera, come in this category, probably the most important organisms are fish. Many species of fish feed readily on black fly larvae; 71 larvae of assorted age have been taken from the stomach of a northern sucker, *Catostomus catostomus* Forster, captured in a sparsely infested creek, and 73 very young larvae from the stomach of a nine-spined stickleback, *Pungitius pungitius* Linnaeus, from a stream which observers had reported to be free from infestation. Larvae have also been found in the stomachs of jackfish, *Esox lucius* (L.), and of the northern wood frog, *Rana sylvatica cantabrigensis* Baird. Finally, and perhaps of the greatest immediate importance, is the possible influence on other animals, particularly fish, of economic value. This, too, of course may be a direct influence at any stage of the life-history, or an indirect influence through the food supply.

Toxicity to Fish

At Churchill in 1948 a number of tests were made by P. M. Jackin, of the Defence Research Board, of the effects of three of the more important insecticides, DDT and BHC emulsion concentrates and parathion wettable powder, on local species of fish in tanks and cages. The species of fish used were: jackfish, *Esox lucius:* northern sucker, *Catostomus catostomus:* lake trout, *Cristivomer mamaycush* Walbaum; arctic grayling, *Thymallus signifer* Richardson; brook stickleback, *Eucalia inconstans* Kirtland; and nine-spined stickleback, *Pungitius pungitius:* the last two were treated collectively. All exposures were for 15 minutes. Although the numbers of fish used were rather small, the following tentative conclusions from the results reported* are justified:

Jackfish, suckers, and lake trout did not suffer any visible or permanent injury as adults from single dosages of DDT up to 600 p.p.m.-minutes, 400 times that required for control of black fly larvae; 50 per cent mortality of sticklebacks occurs at about 150 p.p.m.-minutes, and of fry of arctic grayling at about 450 p.p.m.-minutes; sucker fry show no immediate symptoms after 150 p.p.m.-minutes.

Parathion did not kill adult suckers at 150 p.p.m.-minutes, and there was no immediate effect on fry at 250 p.p.m.-minutes. Fifty per cent mortality of sticklebacks occurred at about 900 p.p.m.-minutes.

Gamma BHC had no effect on the fry of suckers at 1.5 p.p.m.-minutes but these became violently active after 6.7 p.p.m.-minutes; 50 per cent mortality of both sticklebacks and of grayling fry occurred at about 20 p.p.m.-minutes.

After treatment with DDT at about 100 p.p.m.-minutes, jackfish began to open and close the jaws rapidly and then to swim violently about the tank. Continued exposure resulted in a comatose condition and in some interference with orientation mechanisms; on being returned to fresh water, however, most of the fish recovered overnight.

Toxicity to other arthropods

As in 1947, samples of the organisms killed or incapacitated in stream treatments were taken by means of screens fixed in the streams at the lower end of the observed stretch. At least one such sample was taken for each of the newly tested active materials. Finer screens (40 mesh) were used than in 1947, and in 1949, an attempt was made to introduce a quantitative element into this phase of the work, screens 6 inches square being used, and the flow through these being measured at the beginning and end of the experiment.

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* Entomological research in northern Canada. Progress Report, 1948, Pt. 2. Def. Res. Bd. Rept. D.R. 19. 1949.

In larvicide experiment 1 at Churchill in 1948 (Table 2), the first test with parathion, a large screen was used; and in a portion of the material from this screen there were the following numbers of insects and other arthropods:

MOLLUSCA		
Gastropoda		
Pulmonata		3
ARACHNIDA		
Acarina		
Hydrachnidae		3
CRUSTACEA		
Branchiopoda		59
Copepoda		22
INSECTA		
Apterygota		
Collembola		
Arthropleona		8
Expoterygota		
Plecoptera		
Nemouridae	nymphs	87
Ephemerida	nymphs	21
Hemiptera		
Corixidae	nymphs	1
Endopterygota		
Coleoptera		
Dytiscidae		3
Trichoptera	larvae	2
Diptera		
Nematocera		
Chironomidae	larvae	5
	pupae	3
	adults	6
Heleidae		
Culicoides sp.		2
Tipulidae		1
Culicidae		
Aedes sp.		1
Simuliidae	larvae	323
	pupae	5
Mycetophilidae		2

In so far as it was possible to determine from a field examination, the crustaceans were all alive at the time of collection, 24 hours after treatment; the insects, however, were dead.

The results of an examination of the quantitative screen samples obtained in 1949 at Whitehorse are summarized in Table 5. With the exception of mature larvae of Simuliidae, specific identification has not been possible. All sufficiently mature black fly larvae were identified to species, but the results did not suggest specific variation in susceptibility to any insecticide. The arachnids were all mites of the family Hydrachnidae, representing 3 species. The Collembola represented 2 genera, *Proisotoma* in the Isotomidae, and *Smynthurus*. Three genera of stoneflies were represented, *Isoperla*, *Alloperla*, and *Nemoura*, there being several species of the last. The mayflies belonged in either the Baetidae or the Ecdyuriidae. The caddis flies showed the greatest diversity for the number of specimens obtained, the following groups being represented; Brachycentridae: *Micrasema*, 2 species; Limnephilidae: 2 species; Rhyacophilidae: *Rhyacophila vibox* Milne; Phryganeidae and Psychomyidae: one species each.

TABLE 5.—THE EFFECTS ON INSECTS AND ARACHNIDS OF TREATMENT OF STREAMS WITH VARIOUS INSECTICIDES, WHITEHORSE, Y.T., 1949

Experiment No.	4	6	8	12	14	16	17	19	22
Insecticide	Dieldrin (wettable powder)	Aldrin (emulsion concentrate)	Aldrin (wettable powder)	Methoxychlor	Trichloro-benzene	BHC (emulsion concentrate)	BHC (emulsion concentrate)	BHC (wettable powder)	Parathion (emulsion concentrate)
Dosage, p.p.m.-min.	7.5	3.0	3.0	7.5	10.8	7.5	15.0	7.5	1.5
Approximate volume of water sampled (1,000 cu. ft.)	19.4	75.0	39.0	13.1	18.0	38.3	17.0	26.0	21.6
No. of specimens taken, dead/alive									
Arachnida	0	0	0	0	2/1	6/1	0	0	0
Insecta									
Collembola	1	0	0	0	0	1	0	1	0
Plecoptera (nymphs)	419/2	4/1	5/2	12/1	0/2	134/6	7/4	7	32
Ephemerida (nymphs)	103/1	4	3/1	27/1	0	107	1	2	15
Trichoptera (larvae)	5	1	1	0/1	0	24/2	0	0	1
Diptera									
Chironomidae (larvae)	2/1	0	3	0/3	3	19/2	7	2/1	7
Chironomidae (pupae)	0	2	4	4	0	4/1	0	1	2
Simuliidae (larvae)	19/1	3/1	4/1	7	0	94/5	5/1	1	40/7

Other groups, not shown in Table 5, were represented by single specimens; these included 2 crustaceans, a species of cyclops and an ostracod; corixid nymphs; other nematocerous larvae; and many specimens obviously not from the aquatic habitat, such as numerous thrips, psyllids, and mycetophilid and lonchopterid flies. The most striking differences between these samples and the samples previously obtained at Churchill were the small amount of material obtained and the virtual absence of Crustacea and Collembola. The very low chloride content of the water may be the principal factor responsible for these differences.

In view of the very varied nature of the streams, comparative deductions from these data are scarcely possible. It appears, however, that none of these insecticides is usefully selective in toxicity to black fly larvae, although crustaceans are apparently resistant to parathion, and 1, 2, 4-trichlorobenzene has a lower general toxicity than any of the other materials.

DISCUSSION

Parathion wettable powder and benzene hexachloride emulsion each have useful ovicidal properties, at least against eggs believed to be those of *Simulium venustum*; but until more is known concerning the oviposition sites and egg-laying habits of other species, it is doubtful whether much practical use can be made of this. F. J. H. Fredeen, in studies of *S. arcticum* in the South Saskatchewan River, has recently shown that eggs of this species are to be found buried in the sand of the stream bed; other species may oviposit above water level.

For controlling the larvae of the great majority of the important Canadian species of black flies, none of the new materials tested compares at all favourably with DDT in oil solution. All the formulations of dieldrin and parathion that were tested, and the wettable powder and oil solutions of benzene hexachloride, give control, at least of certain species, at dosages not much greater than that required for DDT; but none of these exhibits any encouraging selectivity in its effects on stream fauna.

Some toxicity to the pupae of certain species is shown by aldrin wettable powder, by benzene hexachloride and parathion emulsions and wettable powders, and by trichlorobenzene at high dosages. This toxicity, however, decreases rapidly with increasing pupal age, until by the time the pupa is surrounded by an air film it can obviously be affected only by fumigant activity such as that of trichlorobenzene. In practice, it is again doubtful whether any use can be made of these properties, except in special circumstances. If a population has been allowed to develop until a significant proportion has pupated, it might be a satisfactory procedure to spray the vegetation bordering the stream with a DDT emulsion at first emergence to kill adults.

At first sight the results of tests against grown fish are encouraging; newly hatched fry, however, are likely to be far more susceptible, and interference with spawning is a possibility. It is clear that the long-term effects on the arthropod fauna of repeated treatments of streams with any of these chemicals except perhaps trichlorobenzene are likely to be profound. To what extent these effects would influence fish is impossible to predict; a long-term ecological survey of a large stream under such repeated treatments would be needed to determine this point. The statement of Travis (11) that: "Studies. . . .showed that DDT caused no damage to fish or fish foods at 30 to 60 p.p.m." is, however, at variance with findings reported in 1949 (5) and in this paper, unless the exposure time in the studies in question was reduced to the rather impracticable figure of about 1.5 seconds.

It is difficult to estimate the effect which local killing of black fly larvae would have on the human pest problem in an area. This would depend principally, of course, on flight range and flight habits of the species concerned. Though the flight range of *S. arcticum* is known to be considerable, there are some grounds for believing that for many important pests of man the range is much more limited. This question can best be settled by a double approach: direct observation on the flight habits of the species concerned, and field trials of large-scale area control in the larval stage coupled with periodical assessments of adult populations.

Two steps, a long-term ecological study of the influence on the fauna of repeated treatments of streams with DDT, and the accumulation of data on flight range and flight habits of important biting species, are of paramount and immediate importance in the progress of black fly control work.

SUMMARY

Various formulations of DDT, parathion, gamma benzene hexachloride, methoxychlor, aldrin, dieldrin, and 1, 2, 4-trichlorobenzene were tested in the field for toxicity to the larvae and pupae of several species of black flies (Simuliidae). Laboratory tests of parathion suspension and benzene hexa-

chloride emulsion showed that these materials have some ovicidal properties. Against larvae, DDT in oil solution is still by far the most satisfactory insecticide. Parathion; benzene hexachloride; aldrin; and trichlorobenzene at high concentrations show some toxicity to pupae, but the practical value of this and of the ovicidal materials is doubted.

DDT, benzene hexachloride, and parathion were tested on the fry and adults of several species of fish. The absence of any immediate permanent effects at the dosages required to kill black fly larvae is encouraging.

Observations on the effects of all insecticides except DDT on other fauna in the streams treated are recorded. The results do not suggest that the toxicity of any of the materials is more selective to black fly larvae than that of DDT.

A dispenser for the convenient and accurate application of insecticides to streams is described in an appendix, and simplified instructions for its use are given.

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APPENDIX

INSTRUCTIONS FOR THE CONTROL OF BLACK FLIES

Black flies can be controlled by the application of an oil solution of DDT, preferably 10 per cent, to streams in which the larvae (Figure 10) develop. Since these solutions affect many other insects and animals, including fish, it is important that the correct quantity be carefully applied at the proper time and place.

The quantity required is arrived at in the following way. At any convenient point measure the width (W) and average depth (D) of the stream in feet, and its maximum speed of flow (V) in feet per second. The speed can be obtained by timing a fairly large floating object over a measured distance, not less than 10 feet. The float used should not project much above the water, or wind will affect its movement; a piece of wet wood is suitable. If much work is to be done, a pitot tube or a hydrographic flow meter would be worth-while. The quantity of a 10 per cent solution required is then:—

$$17 \times VWD \text{ cc.}$$

This amount can be measured in a graduate, and dribbled into the stream by hand as evenly as possible over a period of 15 minutes. If much work is to be done, however, a dispenser constructed as shown in Figure 8 is useful.

The best time to make this application is immediately before the larvae transform into pupae (Figure 11); in practice the appearance of the first pupae should be carefully watched for, and application should then be made as soon as possible. It is best to apply the insecticide upstream of as much of the infestation as possible, preferably just above a turbulent area in order to improve mixing.

Construction and calibration of a dispenser

A suitable dispenser can be constructed almost entirely from a 10-gallon oil drum and standard pipe fittings. The essential features are the two valves, the two manometer tubes with scale, and provision for changing the orifice plates, which are held in the gasket-type union (L). Leather washers cemented to either side of these plates are a convenience, and a leather gasket under the lid reduces waste in transit. Various modifications of the design shown are possible, to take advantage of locally available material.

Before use, the dispenser requires calibration, preferably with the DDT solution for which it is to be used and at about the same temperature. The tank is filled with this solution strained through 50-mesh screening, and the first valve (N) is opened fully. The fluid then rises to the top of both manometer tubes. As the second valve is opened the level in the second tube falls progressively. It is necessary to measure the delivery rate in cc. per 15 minutes for various pressure differences in the two tubes, so that a curve connecting the two can be drawn as shown in Figure 9. This must be done for each of the three orifice plates.

INSECTICIDE DISPENSER FOR STREAM TREATMENT

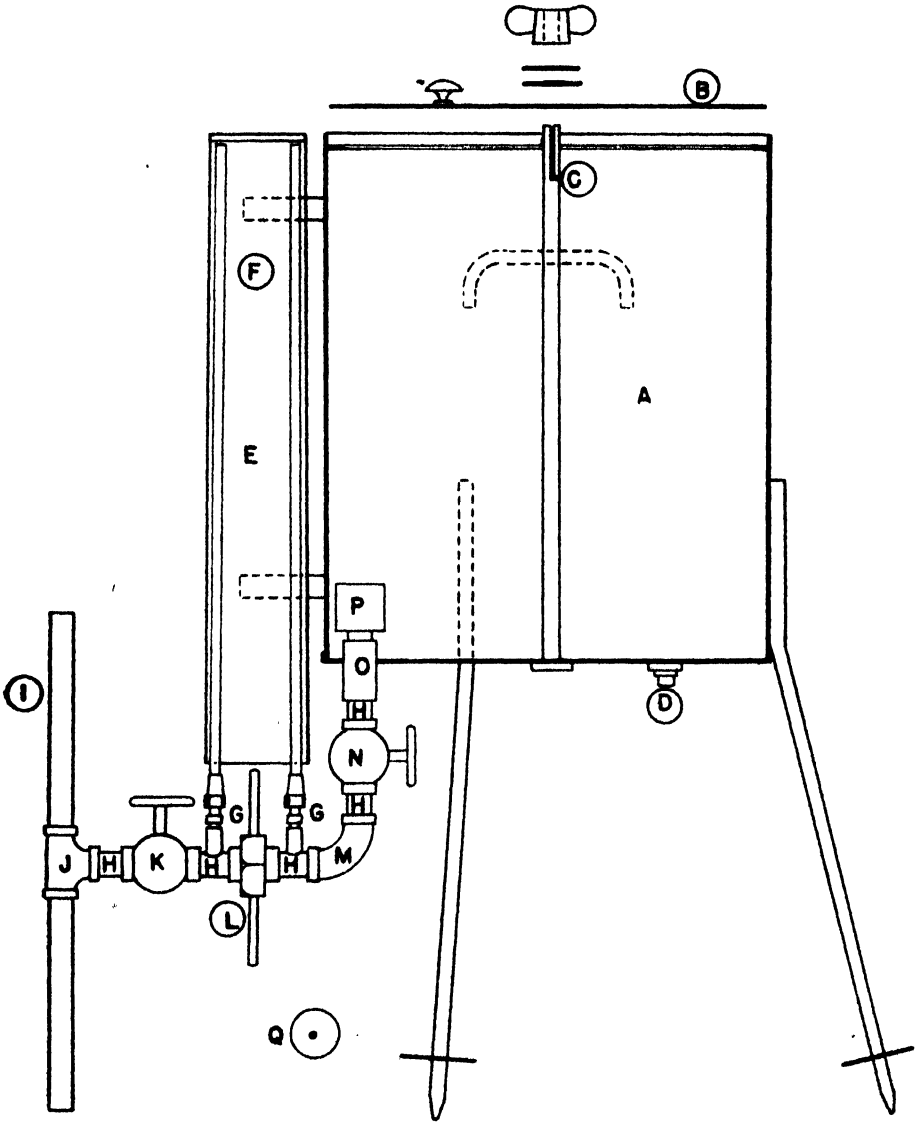

FIGURE 8. Insecticide dispenser for stream treatment. Construction drawing for final design.

A. 10-gallon drum
B. Lid, 1/16" steel
C. ⅜" bolt with air vent
D. Drain plug
E. Centimeter scale board
F. ¼" Saran tubes (2)
G. ⅜" I.P.S. × ¼" Saran Adapter (2)
H. ⅜" × 1½" nipples (5), 2 with ⅜" couplings welded on
I. ⅜" × 7" pipe (2)
J. ⅜" std. tee

K. ⅜" plug disc or needle point brass globe valve
L. ⅜" gasket type union, 2" lugs welded on,
M. ⅜" std. elbow
N. ⅜" composition disc brass globe valve
O. ⅜" × 2" coupling welded in
P. 50-mesh brass screen cylinder 1½" × 1½" soldered to ⅜" nipple
Q. Brass orifice plate ⅛" × 1½" D. (3), drilled 0.020", 3/64", and 9/64"

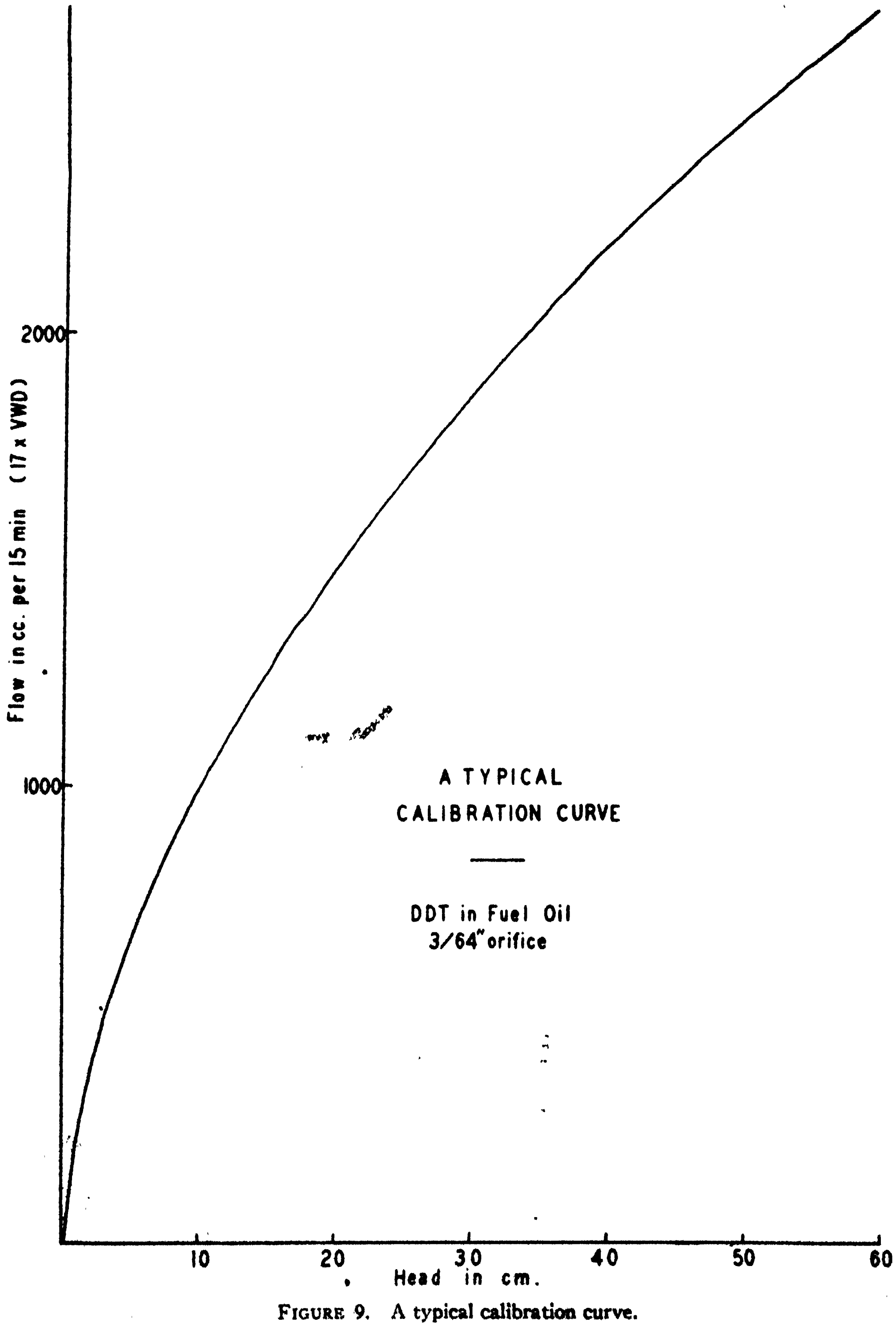

FIGURE 9. A typical calibration curve.

Use of dispenser

The dispenser may be set up on the bed of the stream, or on a bank, bridge, or culvert, so that the delivery pipe will discharge into the water. A short length of rubber hose, three-quarters of an inch in diameter, may be useful to conduct the insecticide. The tank should be levelled from front to rear. This can be done by raising or lowering the rear leg until both manometer pipes give the same reading. The appropriate orifice and pressure difference for any stream can be arrived at by looking up the figure 17 × VWD on the calibration curves. With the correct orifice in position, the second valve (K) is opened until the pressure difference reaches the required figure, and the insecticide is allowed to run for 15 minutes. In treating larger rivers, it may be necessary to adjust the valve K during the the 15 minutes to compensate for the fall in level in the tank.

Rivers with flow up to about 1,000 cubic feet per second can be conveniently treated with this equipment.

VARIATION IN THE SEED SETTING ABILITY OF LADINO WHITE CLOVER[1]

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[Received for publication July 27, 1950]

Environmental conditions in Eastern Canada are not in general favourable to seed production of Ladino clover. Whether this condition is due to lack of adaptation of the plant or to unfavourable environment has not been determined. Nevertheless, one of the main objectives of the Ladino breeding programme instigated in 1947 was to increase seed productivity. At the same time data were recorded from the breeding material with the purpose of investigating whether the range of variation of some of the characters involved in seed productivity was large enough to justify selection.

Very little information is available on the genetical variability of white clover in relation to seed productivity. Differences in the abundance of blossoms were observed between strains by Ahlgren and Sprague (1). Atwood (2, 3) has reported that the number of seeds set in compatible crosses differed between plants. According to Erith (3), many ovules are never fertilized, the number of fully developed seeds in the legume varying from one to seven. The majority of the legumes contain only two or three seeds.

The purpose of this investigation was to determine the influence of climatic environment and genetical variability on seed setting and percentage of seed-bearing florets in Ladino white clover.

MATERIALS AND METHODS

The materials used throughout this investigation came from commercial seed sources. Individual plants were started in the greenhouse in April and transplanted to the field in May. Seedlings were set three feet apart on clay soil. Data were recorded during the first year of growth.

In general four heads of equal maturity were sampled from each plant on a given date. In some cases a random sample of 10 florets was taken from each head to determine seed setting. In other cases all florets were counted and the number of seeds recorded individually. In these investigations, whole heads were used, unless otherwise specified. These determinations were made under field conditions. Nothing was allowed to interfere with honey bee pollination. The farm apiary was located within one mile of the white clover nurseries.

EXPERIMENTAL RESULTS

Effect of environment

Ten spaced plants of Ladino clover were used to measure the effect of climatic environment on seed setting. They were selected among other plants of the 1947 breeding nurseries for their early and profuse flowering. Four heads, from which all but 10 blooming florets were removed, were tagged every week on each of the 10 plants. In general, pollination occurred within a week, except during periods of cold weather. The mature heads were harvested 5 weeks later and the number of seeds was recorded for each individual floret. Storms and foliage density were responsible for the loss of several tagged heads, which could not be found. Some missing data were due to the lack of flower heads on various plants at certain dates. The results obtained are summarized in Table 1.

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TABLE 1.—NUMBER OF SEEDS PER FLORET AND PERCENTAGE OF SEED-BEARING FLORETS IN RELATION TO THE MEAN VALUES OF SOME CLIMATIC FACTORS OBSERVED AT WEEKLY INTERVALS DURING THE SUMMER 1948

Items	Dates of flowering											
	6/23	6/30	7/7	7/14	7/21	7/28	8/4	8/11	8/18	8/25	9/1	9/15
Number of plants	10	10	10	10	10	10	10	10	7	8	8	6
Number of heads	40	39	40	39	39	37	32	35	27	20	30	20
Number of flowers	337	369	387	396	363	328	293	340	232	188	283	194
Per cent seed-bearing florets	85.6	63.8	74.5	85.9	86.8	84.5	84.4	81.3	91.9	91.4	88.4	0.8
Number of seeds per flower	2.0	1.4	1.9	2.4	2.3	2.2	1.9	2.0	2.7	2.5	2.4	0.03
Weekly means of:	Beginning on:											
	6/23	6/30	7/7	7/14	7/21	7/28	8/4	8/11	8/18	8/25	9/1	9/15
Max. temperature	77.4	74.8	79.8	79.4	74.8	80.1	69.7	75.1	79.6	77.8	79.7	60.8
Min. temperature	53.4	52.7	55.1	53.0	56.6	60.6	51.8	52.4	55.6	58.1	53.7	41.1
Rain duration (hours)	4.75	14.00	7.75	4.50	27.50	8.50	22.75	18.00	7.50	16.25	0.00	43.00
Rainfall (inches)	0.56	0.62	0.67	0.34	1.14	0.40	0.89	1.80	0.27	0.67	0.00	2.25
Sunshine (hours)	50.7	59.0	64.2	55.4	39.8	76.2	50.1	59.4	70.8	58.8	65.2	12.2
Bee activity (hours)	65	62	68	71	53	73	48	56	66	54	66	11

The average number of seeds per floret varied during the season from 0.03 to 2.7. Florets pollinated during the period from August 18 to September 1 gave the highest seed sets. Poor seed setting was obtained on the florets pollinated in the early part of July. Flowers blooming in the middle of September produced a small amount of aborted seeds. The very low yield of sound seeds is explained by the occurrence of a killing frost during the process of seed formation. A few seeds in one plant only were matured enough to escape frost damage. The results obtained on September 15 were excluded from the analysis of variance. Differences between the number of seeds per floret observed at different dates were highly significant, with a least significant difference of 0.4 at the 1 per cent level.

The percentage of seed-bearing florets varied from 0.8 per cent to 91.9 per cent according to the date of flowering. It was noted that a variable number of florets did not produce any seed at all dates, due apparently to a lack of pollination. The percentage of seed-bearing florets was particularly low in the early part of July; this explains the low seed setting obtained during that period.

The degree of pollination seems to be an important cause of variation in seed setting during the season. The weekly variations in seed setting are apparently a result of the complex interaction of several climatic factors. High temperatures, little rain of short duration and abundance of sunshine which are favourable to bee activities, tend to increase seed setting, although it is difficult to find any close relationship between seed setting and any climatic factors, with the exception perhaps of rain duration.

Distribution of the number of seeds per floret

A total of 91,481 individual florets were examined in 1948 and 1949 from a large number of plants among unselected populations of Ladino white clover. The frequency distribution for the number of seeds per floret is recorded in Table 2.

On the average 11 per cent of the florets did not produce any seed. The maximum number of seeds per floret was 7, with a frequency of less than 1 per cent. Two or three seeds per floret were the most frequent classes. There were slight changes in class frequencies from year to year and within a season. The greatest changes were observed between individual plants.

Variation between plants

The seed productivity of 131 individual plants of Ladino clover was studied at two different dates: July 8-12 and August 17-18, 1948. Seed setting was measured by two methods. One method consisted in retaining only 10 flowers on each head, at the stage where these flowers were just about to open. These heads were then tagged, and harvested 5 weeks later. In the other method, the whole head was tagged when the lower florets were starting to bloom, and they were harvested after they had reached maturity. Seed counts were made by dissecting each pod individually.

The mean number of seeds per floret was calculated by dividing the total number of seeds by the total number of flowers within each head, and by averaging the results from the four heads at both dates for both methods. Highly significant differences were observed between individual plants. The mean number of seeds per floret ranged from 0.8 to 4.3 with an average of 2.38 seeds per floret. The variation among plants is demonstrated graphically in Figure 1. Plant distribution was normal. From those 131 plants, 2 plants were found with a seed setting rating which is 4 standard deviations removed from the mean.

Significant differences were found in the seed setting values obtained with the two methods of measurement. There was a tendency to underestimate the number of seeds per floret when a sample of 10 florets was used instead of the whole head. The high degree of consistency between the two methods justifies the use of either one to estimate the number of seeds per floret, when comparative results are the main purpose. However preference should be given to the method using whole heads, if small differences are to be detected, because the variance and the coefficient of variability are appreciably lower with the latter method. Significant

TABLE 2.—FREQUENCY DISTRIBUTION OF FLORETS IN RESPECT TO NUMBER OF SEEDS AMONG UNSELECTED POPULATIONS OF LADINO WHITE CLOVER

Dates	Seeds per floret								Number of florets
	0	1	2	3	4	5	6	7	
July 1948	4,600 11.61%	6,198 15.64%	8,543 21.56%	8,815 22.24%	6,594 16.64%	3,173 8.01%	1,311 3.31%	393 0.99%	39,627 100.00%
August 1948	2,937 10.12%	4,810 16.58%	6,896 23.77%	7,209 24.84%	4,814 16.59%	1,786 6.16%	514 1.77%	49 0.17%	29,015 100.00%
1949	2,856 12.51%	3,712 16.25%	5,347 23.41%	5,644 24.71%	3,704 16.22%	1,267 5.55%	300 1.31%	9 0.04%	22,839 100.00%
Total	10,393 11.36%	14,720 16.09%	20,786 22.72%	21,668 23.69%	15,112 16.52%	6,226 6.81%	2,125 2.32%	451 0.49%	91,481 100.00%

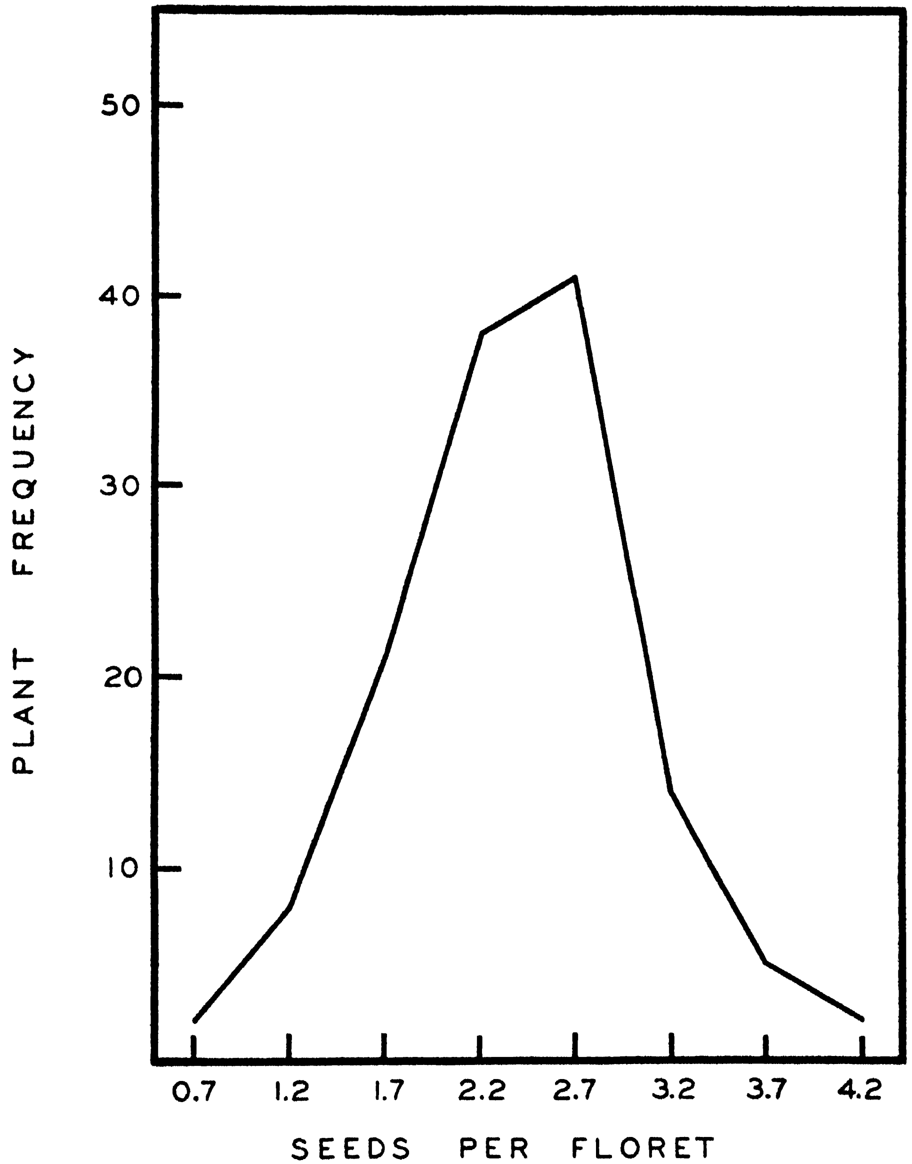

FIGURE 1. Frequency curve of the number of seeds per floret among 131 individual Ladino plants in 1948.

differences between the two dates indicate that a better seed setting was obtained from florets blooming in July than from those blooming in August. These results conflict with those reported in Table 1.

A seed setting determination was made in 1949 from 90 plants of a pure Ladino seed lot supplied by the Oregon Agricultural Experimental Station. The results are shown graphically in Figure. 2. The mean number of seeds per floret ranged from 1.2 to 3.7, with an average of 2.5. Differences between plants were highly signficant.

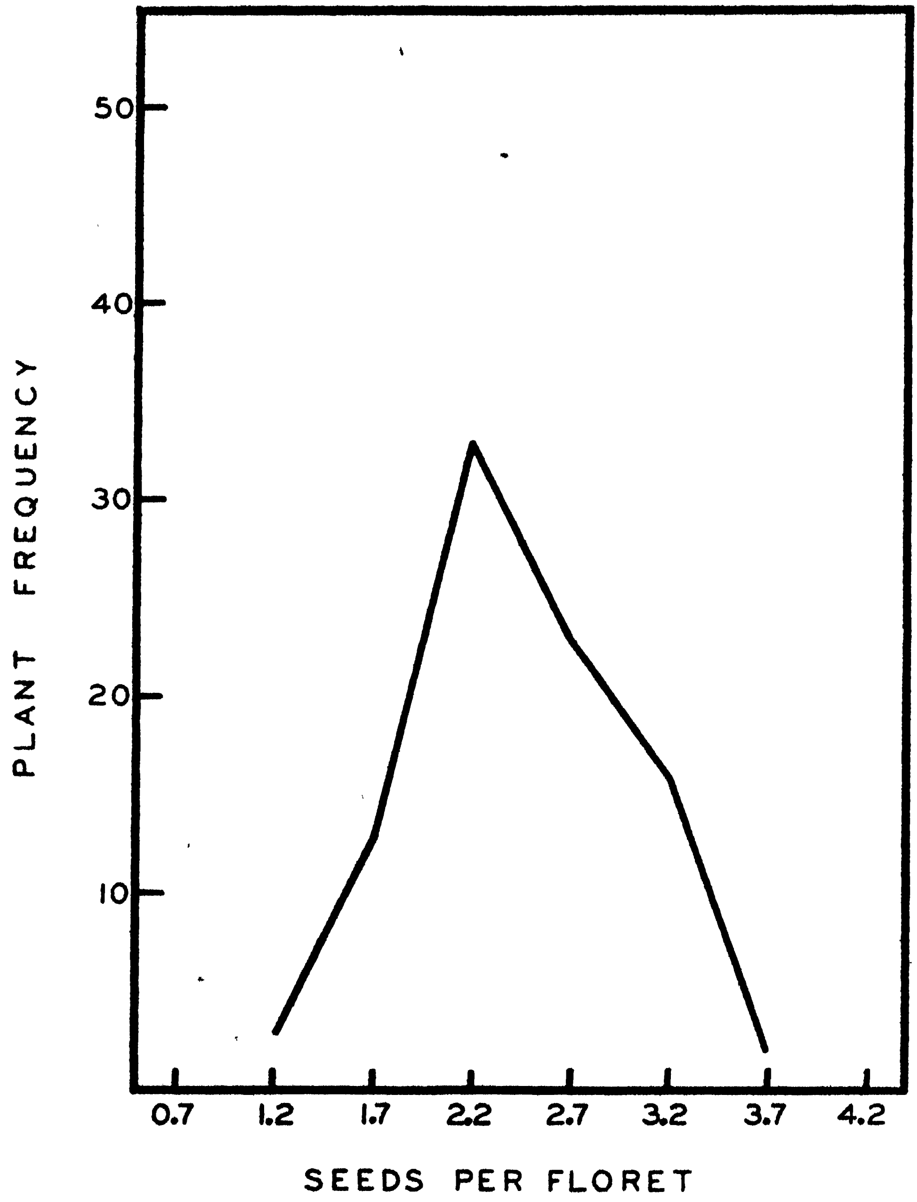

FIGURE 2. Frequency curve of the number of seeds per floret among 90 plants of pure Ladino clover in 1949.

A series of 70 clonal lines were investigated in 1949 for their seed setting ability. The results are summarized graphically in Figure 3. The mean number of seeds per floret ranged from 0.5 to 4.0, with an average of 2.3. Differences in seed setting between clons were highly significant. The frequency distribution of the number of clons in relation to the number of seeds per floret appears to be nearly normal. The clons used in this experiment, with the exception of a few, had been selected for characters other than seed setting.

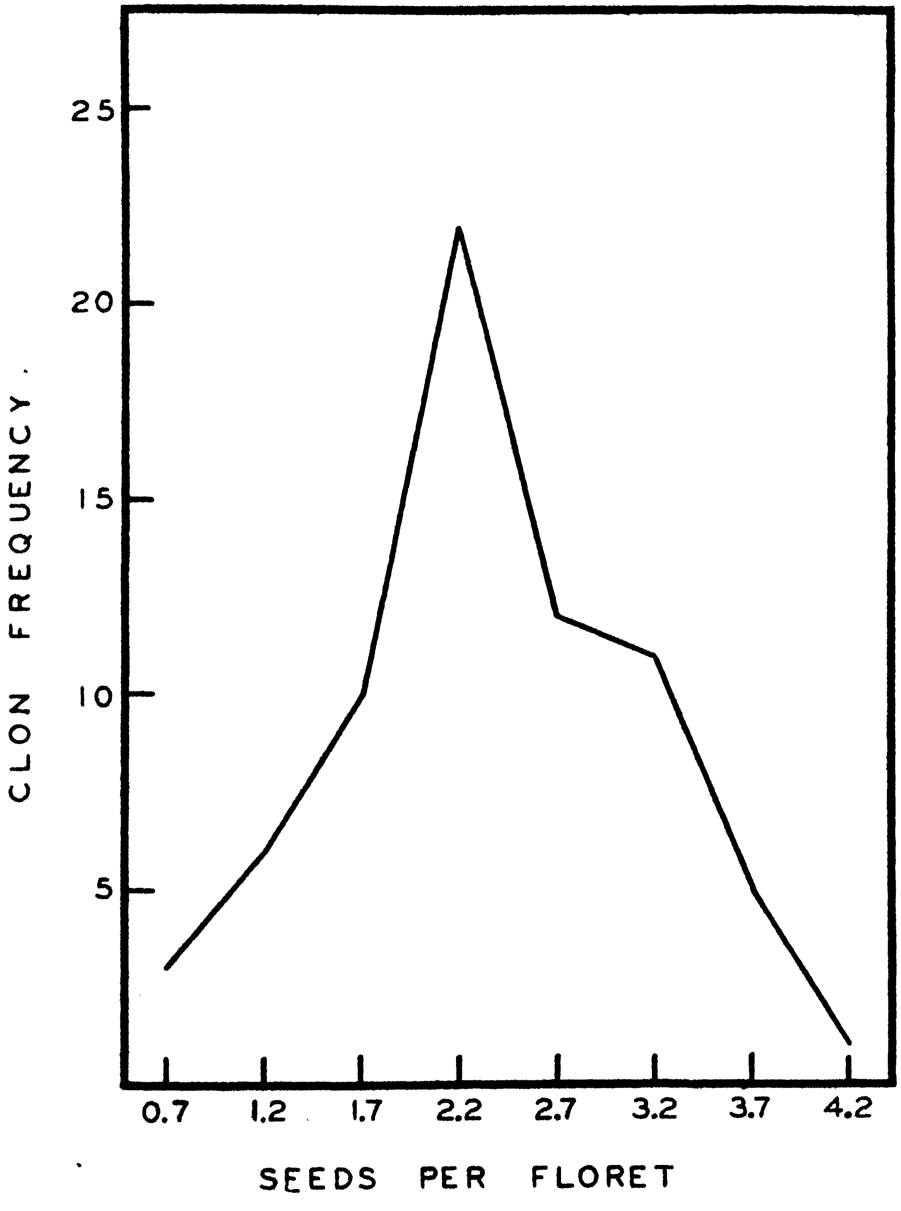

FIGURE 3. Frequency curve of the number of seeds per floret among 70 clons of Ladino clover in 1949.

Seed setting data were available in 1948 and 1949 for 40 of these clons. The 1948 data were collected from seeded single plants, whereas the 1949 data were collected from the vegetatively propagated plants. The coefficient of correlation calculated from the paired means of each clon was found to be highly significant (r=0.727). High seed setting plants in 1948 remained high seed setters in 1949.

It is shown in Table 1 that an average of 89 per cent of the florets produced seeds. The percentage of seed-bearing florets was found to vary with the various plants. In 1948 it ranged from 54.5 per cent to 97.4 per cent. Among the clonal lines investigated in 1949, it ranged from 33.4 per cent to 98.3 per cent. These differences were highly significant. The wide range of variation in the percentage of seed-bearing florets indicates that all plants are not pollinated to the same extent. It is assumed that some white clover plants are more attractive to pollinating insects than others.

A comparison between the percentage of seed-bearing florets in 1948 and 1949 among the paired means of 33 clons gave a correlation coefficient of 0.530, which was highly significant. The individual plants producing a high proportion of unfertilized florets in 1948 also tended to produce a high proportion of unfertilized florets, as clon, in 1949.

The variation among plants or clons in the percentage of seed-bearing florets is reflected in the number of seeds per floret. High seed setting is usually associated with a high percentage of seed-bearing florets, whereas low seed setting is associated with a low percentage of seed-bearing florets. A low percentage of seed-bearing florets in a given plant or clon tends to decrease the number of seeds per floret. In order to eliminate this variation due to unfertilized florets, the total number of seeds for each individual was divided by the total number of seed-bearing florets. This mean number of seeds per seed-bearing floret could be taken as a seed setting rating, assuming that all florets produced seeds. The percentage of seed-bearing florets was significantly correlated with the number of seeds per seed-bearing floret. The 131 plants grown in 1948 gave a correlation of 0.620 and the 70 clons grown in 1949 a correlation coefficient of 0.657. Both were highly significant. When the percentage of seed-bearing florets is high, the number of seeds per seed-bearing floret is also high. If a high proportion of florets remains unfertilized in a given clon, the seed-bearing florets generally produce only a few seeds each, indicating that a large proportion of ovules do not develop into seeds. The degree of pollination influences not only the number of florets which set seeds but also the number of seeds set within each floret.

DISCUSSION

The problem of Ladino seed production in Eastern Canada is rather acute, due to the fact that climatic factors appear to decrease somewhat the activities of pollinating agents. It was assumed that the seed productivity of Ladino white clover could be increased by a better adaptation of the plant through breeding methods. The feasibility of this project was first determined by investigating the range of variation in seed setting among natural populations of Ladino white clover.

The influence of climatic environment on seed setting in Ladino white clover depends to a large extent on the degree of pollination. Pollination of white clover is entirely due to pollinating insects. Any climatic factors likely to change the rate of nectar or pollen-collecting activities of the pollinating agents would result into variations in seed setting.

The wide range of variation in the seed setting ability of Ladino white clover indicates that this character is highly heterozygous. The high degree of heterozygosity is maintained through the cross-pollinating habit of the crop. Variability among Ladino plants offers great opportunities for selection. Inter-crossing of the best seed setting plants should lead to the development of high seed setting strains. Differences between plants as to the degree of pollination suggest the possibility of selecting plants more completely and more effectively pollinated than others. The fact that some plants are more completely pollinated than others indicates that they may be more attractive to pollinating insects. A theoretical increase in seed production of about 50 per cent could be gained through selection for high seed setting only.

The degree of pollination is undoubtedly an important factor conditioning seed setting in white clover. It is suspected that variation between plants in regards to seed setting is not entirely due to differences in the degree of pollination. That is why other factors are now being investigated.

SUMMARY

A rather large number of individual plants and clonal lines of Ladino white clover were examined in 1948 and 1949 in order to determine the mean number of seeds per floret and the percentage of seed-bearing florets. Seed counts were made at weekly intervals with 10 Ladino plants to determine the influence of climatic environment on seed setting.

The mean number of seeds per floret and the percentage of seed-bearing florets was found to vary during the summer.

Wide differences were found between plants or clons as to the number of seeds per floret. The average number of seeds per floret appears to be around 2.4, ranging from 0.5 to 4.3. The percentage of seed-bearing florets also varies from plants to plants, and is an important factor controlling seed setting.

Selection and inter-crossing of high seed setting plants is believed to be a practical means of increasing seed production of Ladino white clover under northeastern conditions.

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THE RELATION OF FOLIAGE ANALYSIS TO KEEPING QUALITY OF McINTOSH AND SPY VARIETIES OF APPLES[1]

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With the loss of a very large proportion of the Canadian export apple market and the consequent occurrence of domestic surpluses, the production of high quality fruit has become all the more essential in order to maintain and to even increase domestic per capita consumption. Fruit quality may be affected by many factors. This paper deals with some phases of plant nutrition and their relationship to certain standards of quality in the McIntosh and Spy varieties of apple.

REVIEW OF THE LITERATURE

Probably the earliest attention paid to the possible effects of plant nutrition and directly to orchard fertilizer practice on apple quality was in the nature of observations by growers themselves. These observations, as well as those of many trained workers, have led to the widely accepted opinion that fruit from trees growing under relatively low nitrogen conditions, though reduced in numbers and small in size, is well coloured, of high quality and keeps well in cold storage. Conversely, fruit from trees growing under high nitrogen conditions, though more numerous and larger in size, is more poorly coloured, has softer flesh and has a shorter storage life.

Wallace (13) states "grass treatment of orchards generally prolongs the storage life of apples both in ordinary and low temperature storage and chemical analysis has shown that this treatment has greatly reduced the nitrogen content of the fruit". Haynes and Archibald (6) have also concluded that low nitrogen is associated with good keeping quality. Wallace (13) has further shown that under a low supply of potassium, fruits remain of dull, immature appearance, they are sub-acid, often sweet or woody and flavourless, shrivel greatly in store, and break down prematurely in low temperature stores.

Information in respect to the levels of phosphorus associated with either good tree vigour and production or quality of fruit is still not very definite. Wallace (13) states "there is still no clear evidence that phosphatic manuring produces direct response in fruit trees, though in several instances it has been shown that indirect effects may follow by stimulating the growth of leguminous cover crops". In California, Leilland (9) found that apple trees failed to respond in growth or production to applications of phosphorus on soils representing extreme cases of phosphorus deficiency with grains and leguminous grasses. Du Toit and Reynke (3) found, however, that high nitrogen applications do not interfere with keeping quality of apples if the phosphorus supply is adequate; that is if a suitable nitrogen-phosphorus ratio exists.

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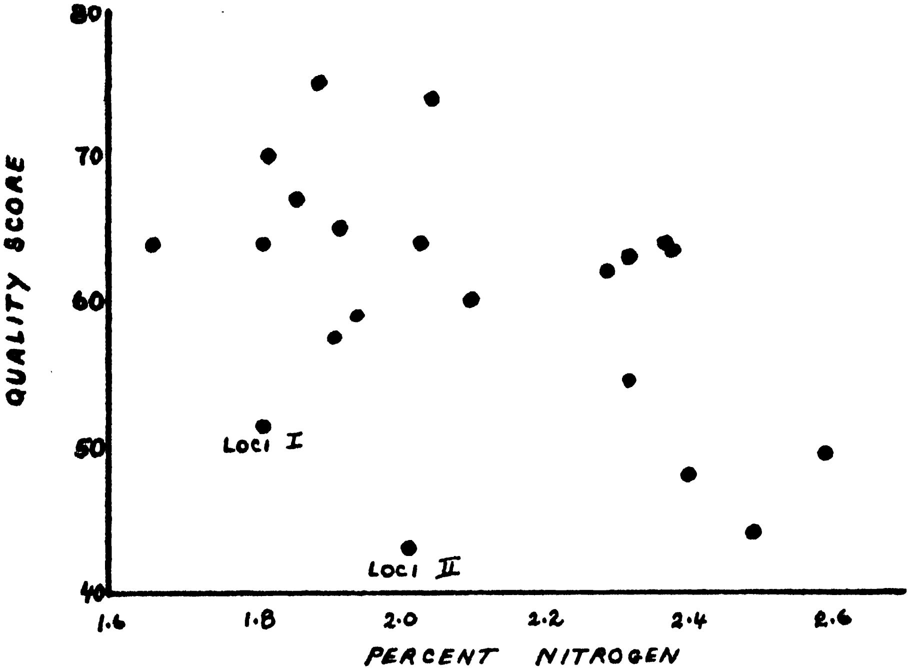

FIGURE 1. Relation between per cent foliage nitrogen and McIntosh storage quality in a group of grower orchards—1948.

Although these references indicate the possible effect of nutrition on apple quality, no definite standards were established which might be employed to advise individual growers regarding fertilizer requirements to produce quality fruit.

Later, Compton *et al.* (2) established leaf colour standards in relation to nitrogen fertilization of the McIntosh variety. A set of colour standards was published indicating the range of leaf colour associated with different levels of leaf nitrogen. It was further determined that trees whose leaves contained less than 1.8 per cent nitrogen in dry weight were low in yield and that trees whose leaves contained more than 2.1 per cent nitrogen yielded maximum crops but fruit of poor colour and generally low in quality.

With the improvement of the more rapid colorimetric methods of plant analysis it was considered that foliage analysis might serve as a basis of service fertilizer recommendations, once standards were established correlated with satisfactory yield and high fruit quality.

METHODS AND MATERIALS

The studies reported here are dealt with under two headings:

1. The relation between foliage analyses for nitrogen, potassium, phosphorus, magnesium and keeping quality of McIntosh and Spy apples grown in a considerable number of different orchards and under a variety of management and fertilizer use.

2. The relation between foliage analyses for nitrogen, potassium, phosphorus, magnesium and keeping quality of McIntosh and Spy apples from trees in a uniform orchard block and receiving specific fertilizer treatments.

Material for the first phase of the study was obtained from the following source: Bearing trees were selected from a number of orchards in a widely dispersed area in the counties of Northumberland and Durham, Ontario. Fertilizer treatment, soil and cultural conditions covered as wide a range as possible and the vigour varied from poor to excessive.

Material used in the second part of these studies was obtained from uniform trees receiving different fertilizer treatments as follows: Ammonium sulphate and superphosphate were employed at four levels in all combinations. Thus, in the data to follow, N_1P_1 represents five pounds of ammonium sulphate and two and one-half pounds of superphosphate and the higher numbers represent multiples of these basic amounts. All trees also received a basic application of one pound and a half of muriate of potash.

COLLECTION AND PREPARATION OF MATERIAL FOR ANALYSIS

Leaf tissue was collected for chemical analysis from each of the trees under test during the second or third week of July. The samples were taken at shoulder level around the circumference of the tree. Only terminal shoots were sampled and the leaves were taken from the middle third of the current year's growth. A sample of one hundred leaves was taken in this manner. The sample was oven-dried at 100°F. for twenty-four hours and was then ground to a fine powder, using at first a Waring Blendor and finally a hammer mill.

ANALYTICAL PROCEDURE

Preparation of Samples

Several methods of wet ashing were tried in order to determine nitrogen and minerals in one sample. However, it was found most satisfactory to dry-ash one sample and to make nitrogen determinations on another.

An air-dry sample equivalent to exactly two grams of dry matter was ashed in an electric muffle furnace for 2½ hours at 550°C. The ash was digested with 4 ml. of 6N hydrochloric acid for one hour on a hot-plate, made up to 400 ml., and filtered.

Potassium Determination

The quick test method of Lindner (10), Peech and English (12) and Wolf (14) were tried but were not satisfactory. Various changes in them failed to give a satisfactory method. Finally a method which was essentially that of Garman (5) was adopted.

Garman makes use of a commercial preparation of sodium cobaltinitrite. However, in this laboratory the products of three different companies failed to give precipitates with micro-quantities of potassium. The product of a fourth company was usable but different lots varied greatly in sensitivity. After numerous trials a reagent of considerable sensitivity and of very uniform performance was evolved. Since the details of its preparation are not immediatley available (7) they are repeated here, as follows:—

(a) 25 grams of cobalt nitrate (hydrated) were dissolved in 50 ml. of water and 16.0 ml. of glacial acetic acid added.

(b) 90 grams of sodium nitrite were dissolved in 150 ml. of water. To *(a)* was added all of *(b)* and the mixture was aerated at the water pump for six hours and filtered. The reagent was stable in the refrigerator for at least a month. It was aerated for a few minutes each day before use. Best results were obtained when the potassium did not exceed 0.8 mg. Results were within an average range of ±4 per cent when compared with quantitative results obtained by the method of McCance and Shipp (11).

Magnesium Determination

The titan yellow method of Peech and English (1) was used with results agreeing with about ±10 per cent of determinations by quantitative methods.

Phosphorus Determination

Silica is not removed in the preparation of the samples. Consequently the method of King (8) was adopted and found very satisfactory.

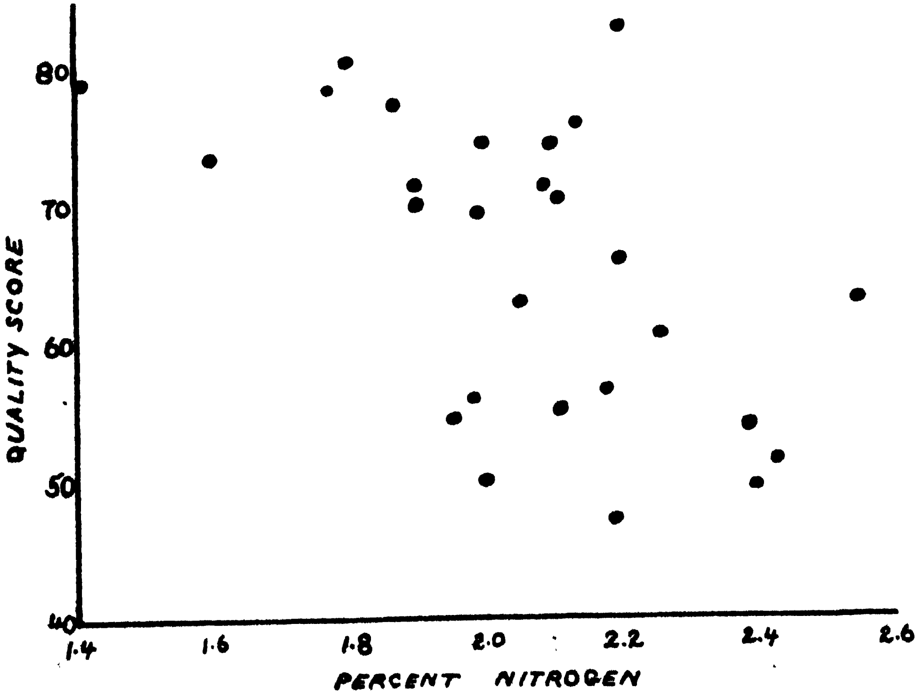

FIGURE 2. Relation between per cent foliage nitrogen and McIntosh storage quality in a group of grower orchards—1949.

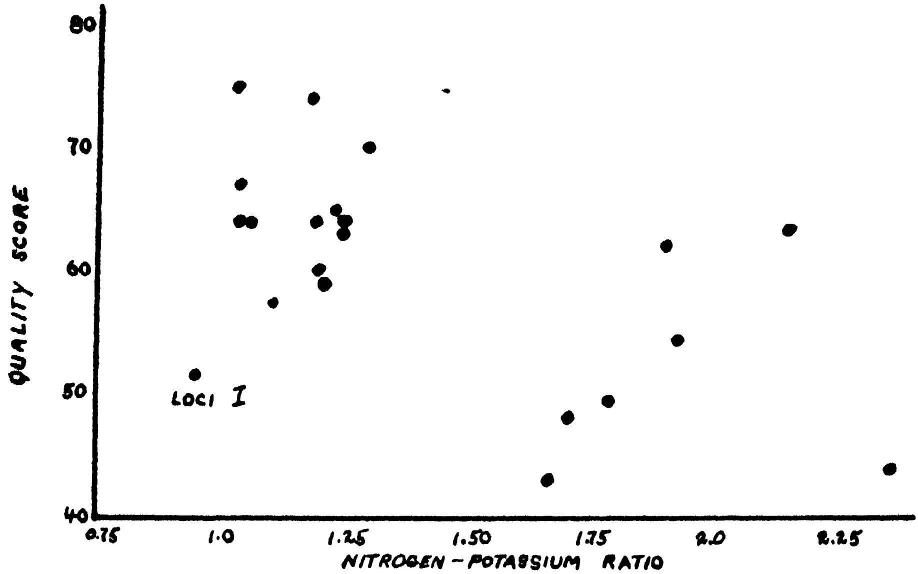

FIGURE 3. Relation between the nitrogen-potassium ratio and McIntosh storage quality in a group of grower orchards—1948.

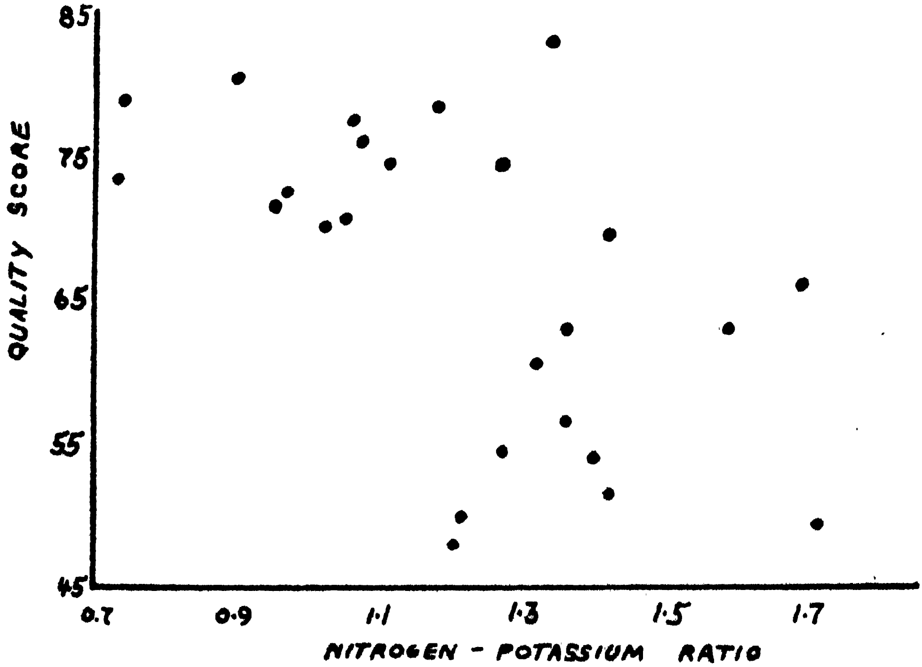

FIGURE 4. Relation between the nitrogen-potassium ratio and McIntosh storage quality in a group of grower orchards—1949.

Nitrogen Determination

Nitrogen was determined by the ordinary Kjeldahl procedure or by a nesslerization method which has proven very rapid and has given results in good agreement with the Kjeldahl method.

An 0.5 gm. sample was placed in a 125 ml. erlenmeyer flask with 10 ml. of the digestion mixture of Campbell and Hanna (1). Glass beads were added, the flask heated gently until foaming occurred, and the flame withdrawn until foaming ceased. Boiling was continued until the solution became a clear, pale green, then about two minutes longer—usually a total time of ten to twelve minutes. The digest was diluted after cooling to 100 ml., and a 10 ml. aliquot further diluted to 100 ml. A 1 ml. aliquot was measured into a colorimeter tube, 0.5 ml. of Rochelle salt solution (50 grams in 100 ml. of water) added, and the volume made up to 6 ml. After mixing, 1 ml. of Nessler's reagent—the reagent of Folin and Wu (4) diluted 1 to 1 with water—was added, the whole mixed and an immediate reading made with a blue filter. Comparison with the Kjeldahl method gave results with ±4 per cent, and an average deviation of ±2 per cent.

Collection of Fruit for Storage

Fruit was collected around the circumference of the tree in the same manner as the leaf tissue. This fruit was collected as close as possible to the date at which the grower picked the rest of the orchard. Maturity readings as indicated by the iodine starch test were made at the time of picking. The fruit was placed in cold storage at 32°F., generally within 48 hours after picking.

Methods Used in Evaluating Keeping Quality of Fruit

While the results herein reported are confined to studies conducted in 1948 and 1949, similar studies were also carried out in 1946 and 1947. During the first year of the test the storage quality was based in the case

TABLE 1.—QUALITY EVALUATIONS

Date..............................Sample..........................

		Values	*Max. Score*
(1) Flavour (aroma-acidity)	0 – 10	very poor	30
	10 – 20	poor–medium	
	20 – 30	medium–very good	
(2) Texture (juiciness–mealiness)	0 – 10	very mealy	30
	10 – 20	mealy	
	20 – 30	mellow crisp	
(3) Appearance			
blush	0 – 10	very poor–poor	30
colour quality	10 – 20	poor–medium	
blemish	20 – 30	medium–very good	
(4) Miscellaneous			
hardness			3
greasiness			3
gr. colour		yellow = max. score	3

TABLE 2.—KEEPING QUALITY OF MCINTOSH APPLES GROWN UNDER A VARIETY OF CONDITIONS IN RELATION TO FOLIAGE ANALYSIS EXPRESSED AS PERCENTAGE OF DRY MATTER

Orchard No.	1948						1949					
	Quality	Spoilage	N%	P%	K%	Mg%	Quality	Spoilage	N%	P%	K%	Mg%
1	—	—	—	—	—	—	83.0	28	2.22	0.19	1.65	0.17
2	64	21	2.03	0.19	1.70	0.17	80.5	70	1.80	0.18	2.00	0.18
3	—	—	—	—	—	—	79.0	3	1.41	0.17	1.90	0.22
4	64	0	1.81	0.17	1.70	0.24	78.5	78	1.77	0.17	1.50	0.23
5	75	7	1.89	0.21	1.70	0.24	77.5	65	1.87	0.19	1.75	0.34
6	64	86	1.66	0.34	1.60	0.26	76.0	120	2.14	0.34	2.00	0.18
7	54.5	74	2.32	0.18	1.20	0.28	74.5	21	2.00	0.20	1.80	0.32
8	63.5	8	2.38	0.17	1.10	0.27	74.5	70	2.10	0.19	1.65	0.30
9	67	23	1.86	0.33	1.65	0.34	73.5	26	1.60	0.35	2.20	0.22
10	74	26	2.13	0.26	1.80	0.25	72.5	119	2.09	0.22	2.15	0.28
11	—	—	—	—	—	—	71.5	20	1.90	0.19	2.00	0.27
12	—	—	—	—	—	—	70.5	98	2.11	0.23	2.00	0.32
13	63	23	2.32	0.16	1.85	0.18	70.0	20	1.90	0.22	1.85	0.20
14	—	—	—	—	—	—	69.5	153	1.99	0.26	1.40	0.26
15	62	15	2.29	0.19	1.20	0.28	66.0	110	2.20	0.19	1.30	0.35
16	64	0	2.37	0.22	1.90	0.23	63.0	64	2.55	0.23	1.60	0.34
17	49.5	27	2.59	0.21	1.45	0.24	63.0	5	2.05	0.18	1.50	0.30
18	—	—	—	—	—	—	60.5	97	2.26	0.17	1.70	0.20
19	65	57	1.92	0.14	1.55	0.26	56.5	16	2.18	0.17	1.60	0.31
20	43	58	2.01	0.16	1.20	0.23	56.0	2	1.98	0.27	1.20	0.27
21	—	—	—	—	—	—	55.0	47	2.11	0.21	1.90	0.23
22	—	—	—	—	—	—	54.5	124	1.95	0.20	1.45	0.27
23	—	—	—	—	—	—	54.0	117	2.39	0.19	1.70	0.30
24	48	74	2.40	0.18	1.40	0.30	51.5	133	2.43	0.25	1.70	0.30
25	59	87	1.94	0.19	1.60	0.23	50.0	160	2.00	0.24	1.65	0.30
26	44	0	2.49	0.18	1.05	0.30	49.5	27	2.40	0.22	1.40	0.30
27	60	20	2.10	0.13	1.75	0.23	47.0	117	2.09	0.20	1.75	0.27
28	70	23	1.82	0.17	1.40	0.24						
29	57.5	12	1.91	0.15	1.70	0.20						
30	51.5	124	1.81	0.31	1.90	0.21						

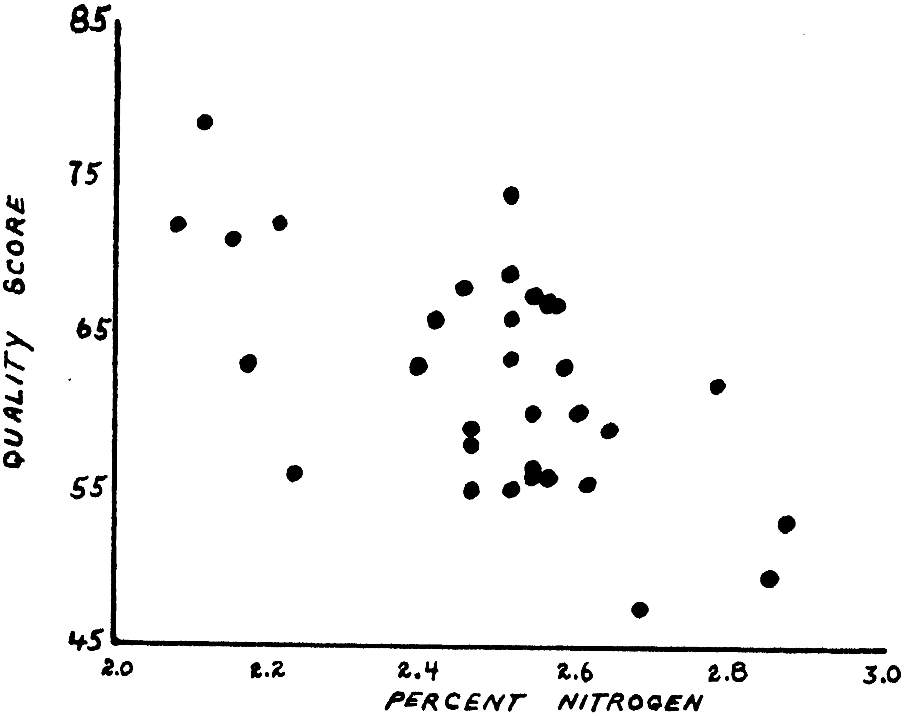

FIGURE 5. Relation between per cent foliage nitrogen and McIntosh storage quality in a uniform orchard block—1948.

of the McIntosh variety on the core flush index, occurring in April after the fruit had been removed from cold storage and placed at 70°F. for one week. While the appearance of core flush may be accepted as an expression of approaching physiological senility it is only after an extended period in storage that samples may be evaluated by this index. In respect to providing the market with apples of acceptable quality the variety should be marketed long before this period. It was therefore considered that other indexes of quality such as general appearance, texture of flesh, flavour, etc., might have more influence on the estimation of quality by the buying public. With this in mind the quality score in Table I was adopted, while the occurrence of core flush in McIntosh and bitter-pit in Spy was retained as the main constituent of the spoilage index.

RESULTS

I. The relation between foliage analysis and the keeping quality of McIntosh and Spy apples grown under a variety of conditions.

The results of foliage analysis, storage quality and spoilage index of the McIntosh variety for 1948 and 1949 are given in Table 2. In both years there were definite negative correlations between the nitrogen content of the foliage and storage quality of the fruit (Figures 1 and 2). In 1948 the correlation coefficient between nitrogen content and fruit quality was $r = -.6153^{**}$ and in 1949, $r = -.374^{*}$. Referring to Figure 1, the position

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** Two asterisks following a coefficient of correlation indicate odds greater than 99 : 1 (highly significant).
* One asterisk represents odds between 99 : 1 and 19 : 1 (significant).

TABLE 3.—KEEPING QUALITY OF SPY APPLES GROWN UNDER A VARIETY OF CONDITIONS IN RELATION TO FOLIAGE ANALYSIS EXPRESSED AS PERCENTAGE OF DRY MATTER

Orchard No.	1948						1949					
	Quality Score	Spoilage Index	N%	P%	K%	Mg%	Quality Score	Spoilage Index	N%	P%	K%	Mg%
1	45.5	0	2.40	0.16	2.05	0.19	85	17	2.1	0.21	2.15	0.20
2	—	—	—	—	—	—	84.5	28	—	—	—	—
3	71	4	2.16	0.18	1.25	0.23	75.0	0	2.3	0.21	1.55	0.28
4	—	—	—	—	—	—	74.0	12	—	—	—	—
5	—	—	—	—	—	—	72.5	0	—	—	—	—
6	62	161	1.79	0.23	1.85	0.22	72.5	43	2.32	0.26	2.60	0.23
7	65	3	2.43	0.20	1.80	0.22	72.5	33	2.40	0.21	2.00	0.25
8	76	21	2.29	0.25	1.80	0.33	72.0	68	2.01	0.23	1.80	0.21
9	65	11	2.23	0.35	1.95	0.28	70.0	8	2.10	0.17	1.60	0.28
10	78	20	2.69	0.22	1.75	0.30	67.0	4	2.20	0.18	1.80	0.37
11	—	—	—	—	—	—	62.0	5	2.30	0.23	2.15	0.27
12	81.5	67	1.91	0.34	1.95	0.22	58.5	13	2.64	0.53	2.10	0.19
13	65.5	16	2.23	0.30	1.70	0.28	—	—	—	—	—	—
14	68.0	14	2.23	0.31	1.75	0.26	48.5	2	2.58	0.25	1.80	0.25
15	59.5	46	2.23	0.34	2.00	0.17	—	—	—	—	—	—

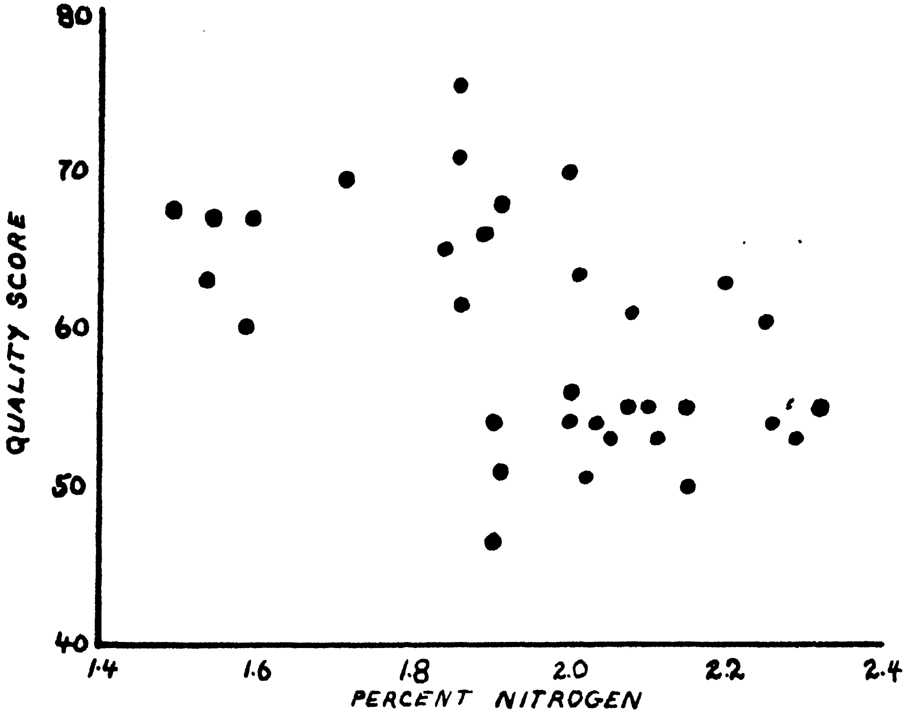

FIGURE 6. Relation between per cent foliage nitrogen and McIntosh storage quality in a uniform orchard block—1949.

in the graph of locus one and two may be accounted for by factors other than the nitrogen content of the foliage. Locus one represents a very closely planted orchard which had been lightly pruned so that quality, especially colour, was low due to poor penetration of light. Locus two represents an orchard which had a particularly low level of potassium in the foliage and it is considered that the supply of this element was the main factor in the quality score.

In 1948 it is apparent that when nitrogen alone is considered, the quality of the fruit is reduced if the nitrogen level in the foliage is greater than 2.0 to 2.1 per cent. The 1949 results largely confirm this relationship.

If the nitrogen values are divided into two categories, below and above 2.1 per cent, there is no correlation in 1948 with nitrogen below this point and a negative correlation with quality above this level of nitrogen in both years (1948 r= −.8124**; 1949 r= −.4419*).

The spoilage index which is derived from the amount and intensity of core flush did not show any constant relationship either with the levels of nitrogen in the foliage or with the quality score of the stored fruit. This fact is apparent on examination of the data for the two years.

In both 1948 and 1949 there was a positive trend between potassium levels in the foliage up to 1.7 per cent of dry matter and increased quality of the fruit. Whereas the single effects of nitrogen and potassium both exert an effect, such single effects are somewhat modified by the ratio of nitrogen to potassium (Figures 3 and 4). In both years fruit from trees

TABLE 4.—EFFECT OF FERTILIZER APPLICATIONS ON FRUIT QUALITY AND FOLIAGE NUTRIENT LEVELS IN 1948 AND 1949 EXPRESSED AS PERCENTAGE OF DRY MATTER

Treatment	McIntosh—1948						McIntosh—1949					
	N%	P%	K%	Mg%	N/K Ratio	Quality Score	N%	P%	K%	Mg%	N/K Ratio	Quality Score
N_1	2.21	0.25	1.82	0.22	1.21	64.4	1.67	0.23	1.68	0.21	0.99	64.8
N_2	2.53	0.21	1.57	0.24	1.61	53.2	1.98	0.18	1.51	0.25	1.29	59.5
N_3	2.62	0.21	1.44	0.24	1.82	53.0	2.03	0.17	1.45	0.26	1.39	58.0
N_4	2.54	0.20	1.43	0.24	1.78	55.4	2.12	0.17	1.42	0.28	1.49	56.9
P_1	2.49	0.21	1.60	0.22	1.59	58.7	1.99	0.19	1.58	0.23	1.27	55.3
P_2	2.45	0.21	1.49	0.24	1.67	53.6	1.96	0.18	1.51	0.26	1.30	60.6
P_3	2.54	0.22	1.58	0.23	1.63	57.9	1.97	0.18	1.48	0.25	1.34	61.2
P_4	2.45	0.22	1.56	0.25	1.58	53.4	1.84	0.20	1.48	0.26	1.25	62.5

Treatment	Spy—1948						Spy—1949					
	N%	P%	K%	Mg%	N/K Ratio	Quality Score	N%	P%	K%	Mg%	N/K Ratio	Quality Score
N_1	2.24	0.29	1.86	0.22	1.20	No Crop	1.85	0.30	1.80	0.22	1.03	76.8
N_2	2.55	0.23	1.60	0.23	1.59		1.98	0.23	1.67	0.23	1.18	71.2
N_3	2.71	0.22	1.63	0.23	1.66		2.18	0.20	1.66	0.24	1.32	60.1
N_4	2.81	0.21	1.51	0.24	1.86		2.18	0.19	1.57	0.22	1.38	62.1
P_1	2.46	0.25	1.66	0.22	1.48		2.00	0.25	1.70	0.22	1.19	67.4
P_2	2.66	0.22	1.62	0.24	1.64		2.02	0.21	1.64	0.25	1.24	68.3
P_3	2.64	0.24	1.67	0.22	1.52		2.06	0.22	1.65	0.23	1.23	69.7
P_4	2.62	0.25	1.66	0.23	1.58		2.12	0.24	1.66	0.23	1.28	65.0

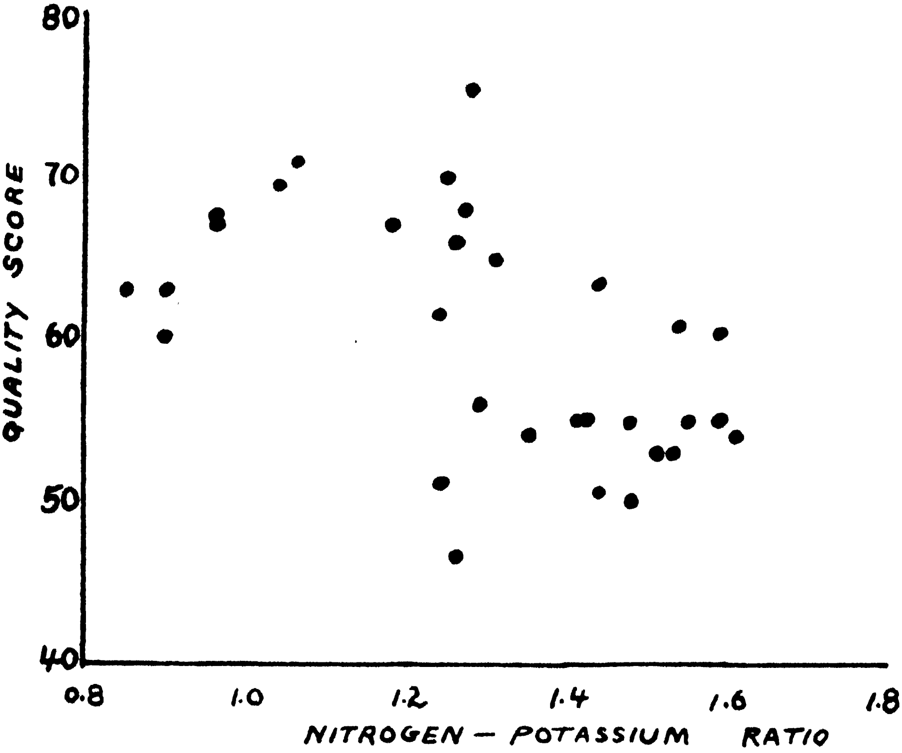

FIGURE 7. Relation between nitrogen-potassium ratio and McIntosh storage quality in a uniform orchard block—1949.

whose foliage had a nitrogen-potassium ration of not more than 1.25 had a significantly higher quality score than those where the nitrogen-potassium ratio was above this figure.

Nitrogen above the 2.1 per cent level would have much more effect in reducing fruit quality if the potassium content was also low and even this level of nitrogen might prove excessive under such conditions.

No relationship was found between the phosphorus content of the foliage and the quality score or spoilage index in either 1948 or 1949.

Results with the Spy Variety

The results of the foliage analysis, storage quality and spoilage index of the Spy variety are recorded in Table 3. No definite relationships were established between any of the elements determined in the foliage and fruit quality, although in 1949 the two samples having the lowest quality score had the highest nitrogen content in the foliage.

II. The relation between foliage analysis and the keeping quality of McIntosh and Spy varieties of apples from trees in a uniform orchard block receiving specific fertilizer treatments.

In addition to the direct relationships between foliage analysis of individual trees and storage quality of the fruit the effect of fertilizer applications on the foliage constituents and fruit quality is of interest and the pertaining data are recorded in Table 4.

TABLE 5.—KEEPING QUALITY OF MCINTOSH APPLES FROM A UNIFORM ORCHARD BLOCK IN 1948 AND 1949 IN RELATION TO FOLIAGE ANALYSIS EXPRESSED AS PERCENTAGE OF DRY MATTER

Treatment	1948						1949					
	Quality Score	Spoilage Index	N%	P%	K%	Mg%	Quality Score	Spoilage Index	N%	P%	K%	Mg%
N_1P_1	67	230	2.21	0.25	1.75	0.21	60	155	1.58	0.23	1.75	0.20
	69	81	2.51	0.23	2.10	0.16	53	216	2.05	0.21	2.00	0.16
N_1P_2	64	84	—	—	—	—	71	135	1.86	0.21	1.75	0.18
	67	92	2.08	0.24	1.90	0.23	63	121	1.53	0.25	1.80	0.25
N_1P_3	66	95	2.15	0.26	1.85	0.22	69.5	126	1.71	0.24	1.65	0.24
	73.5	34	2.11	0.23	1.75	0.22	67.5	99	1.48	0.22	1.55	0.19
N_1P_4	51	110	2.23	0.24	1.80	0.27	67	116	1.59	0.23	1.65	0.26
	58	66	2.17	0.30	1.65	0.26	67	78	1.54	0.26	1.30	0.26
N_2P_1	63	113	2.46	0.21	1.65	0.18	46.5	108	1.90	0.16	1.50	0.19
	54	117	2.64	0.20	1.65	0.22	70	128	2.00	0.17	1.60	0.22
N_2P_2	63	136	2.45	0.22	1.50	0.26	66	111	1.89	0.17	1.40	0.27
	33	205	2.54	0.22	1.50	0.25	61.5	118	1.86	0.17	1.50	0.28
N_2P_3	61	132	2.51	0.21	1.60	0.22	55	154	2.32	0.19	1.65	0.29
	55	186	2.54	0.22	1.50	0.24	55	88	2.10	0.19	1.35	0.22
N_2P_4	54	51	2.43	0.25	1.60	0.27	68	131	1.91	0.19	1.50	0.30
	42.5	138	2.68	0.20	1.60	0.28	54	76	1.90	0.21	1.65	0.29
N_3P_1	50.5	138	2.61	0.22	1.40	0.28	—	—	2.20	0.18	1.50	0.25
	50	126	2.51	0.22	1.48	0.21	50.6	53	2.02	0.18	1.40	0.31
N_3P_2	51	49	2.54	0.20	1.50	0.23	50.0	75	2.15	0.16	1.45	0.25
	51	76	2.56	0.21	1.30	0.26	55.0	117	2.15	0.17	1.45	0.28
N_3P_3	57	184	2.78	0.21	1.65	0.22	56	133	2.00	0.18	1.55	0.23
	44.5	90	2.85	0.20	1.40	0.25	65	136	1.84	0.15	1.40	0.26
N_3P_4	58	184	2.58	0.22	1.40	0.25	75.5	107	1.86	0.19	1.45	0.25
	62.5	77	2.54	0.21	1.40	0.25	54.0	189	2.00	0.19	1.40	0.26
N_4P_1	55	53	2.60	0.20	1.42	0.23	54.0	143	2.03	0.19	1.50	0.31
	61	112	2.41	0.21	1.35	0.27	53.0	127	2.11	0.18	1.40	0.22
N_4P_2	62	126	2.57	0.22	1.50	0.27	63.0	98	2.20	0.17	1.50	0.27
	58	47	2.39	0.20	1.25	0.26	55.0	84	2.07	0.16	1.30	0.30
N_4P_3	48	163	2.87	0.21	1.48	0.25	60.5	119	2.23	0.16	1.40	0.30
	58.5	78	2.51	0.20	1.40	0.24	61.0	85	2.08	0.15	1.35	0.29
N_4P_4	50	162	2.46	0.19	1.60	0.22	51.0	136	1.91	0.16	1.50	0.30
	51	88	2.54	0.20	1.45	0.24	63.5	129	2.01	0.15	1.40	0.30

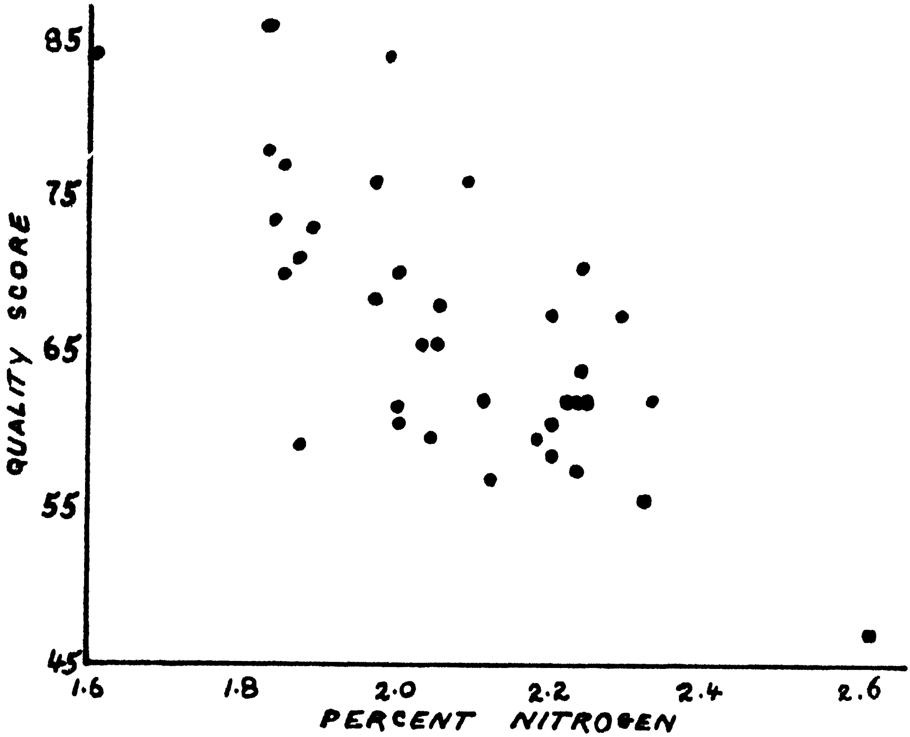

FIGURE 8. Relation between per cent foliage nitrogen and Spy storage quality in a uniform orchard block—1949.

It will be noted that in both varieties progressive increments of ammonium sulphate have brought about a steady increase of nitrogen in the foliage. This increase of nitrogen content is accompanied by a considerable decrease of potassium and phosphorus and increase of magnesium. This increase in nitrogen content and the widening of the N/K ratio is associated with a decrease in the fruit quality score. In the case of McIntosh the first additional increment of ammonium sulphate (10 lb. per tree) has brought about the largest decrease in quality, while in the Spy variety the largest decrease does not take place in 1949 until an application of fifteen pounds per tree, although the N/K ratio is approximately the same in both cases. The significance of maintaining an adequate potassium supply with high nitrogen feeding should be emphasized. Progressive increments of superphosphate had no consistent effect upon the phosphorus, nitrogen or potassium content of the foliage or quality score of the fruit.

The results of the foliage analysis, quality score and spoilage index for individual trees of the McIntosh variety are recorded in Table 5. In both years there were definite negative correlations between the nitrogen content of the foliage and storage quality of the fruit. (Figures 5 and 6). In 1948 the correlation coefficient between nitrogen content and fruit quality was r= −.5762** and in 1949 r= −.5173**. In an examination of Figures 5 and 6 it will be apparent that the quality of the fruit was reduced if the nitrogen level in the foliage was greater than 2.0 to 2.1 per cent. This result is similar to that obtained in the first phase of this study.

TABLE 6.—KEEPING QUALITY OF SPY APPLES FROM A UNIFORM ORCHARD BLOCK IN 1949 IN RELATION TO FOLIAGE ANALYSIS EXPRESSED AS PERCENTAGE OF DRY MATTER

Treatment	Quality Score	Bitter Pit Index	N%	P%	K%	Mg%
N_1P_1	84.0	14	1.61	0.34	2.10	0.25
	68.5	9	1.97	0.29	2.00	0.22
N_1P_2	70.0	19	1.85	0.29	1.55	0.23
	73.0	6	1.89	0.25	1.75	0.27
N_1P_3	78.0	18	1.83	0.36	1.85	0.17
	84.0	26	1.99	0.21	1.55	0.20
N_1P_4	86.0	23	1.83	0.34	1.80	0.21
	71.0	3	1.87	0.33	1.80	0.23
N_2P_1	77.0	1	1.85	0.34	1.70	0.24
	59.0	0	1.87	0.23	1.60	0.20
N_2P_2	76.0	3	1.97	0.21	1.65	0.27
	—	—	—	—	—	—
N_2P_3	70.0	7	2.00	0.21	1.80	0.21
	76.0	6	2.09	0.20	1.70	0.24
N_2P_4	73.5	17	1.84	0.22	1.65	0.23
	67.5	25	2.29	0.20	1.60	0.25
N_3P_1	62.0	23	2.23	0.21	1.55	0.23
	59.5	3	2.04	0.20	1.65	0.23
N_3P_2	61.5	17	2.00	0.19	1.80	0.23
	70.5	35	2.24	0.20	1.40	0.25
N_3P_3	65.5	4	2.05	0.20	1.60	0.24
	58.5	8	2.20	0.19	1.80	0.27
N_3P_4	57.0	1	2.12	0.21	1.80	0.24
	47.0	5	2.61	0.20	1.70	0.27
N_4P_1	62.0	5	2.23	0.20	1.55	0.22
	67.5	10	2.20	0.20	1.50	0.19
N_4P_2	68.0	1	2.05	0.18	1.60	0.24
	59.5	0	2.18	0.21	1.75	0.26
N_4P_3	62.0	4	2.11	0.20	1.70	0.25
	64.0	6	2.24	0.20	1.45	0.25
N_4P_4	60.5	0	2.20	0.21	1.65	0.20

In the 1948 results there is also a progressive increase in quality with levels of potassium up to 1.7 per cent of dry matter. As in the first phase of this study single effects of nitrogen and potassium were somewhat modified by the ratio of nitrogen to potassium. An examination of Figure 7 shows that as the ratio of nitrogen to potassium increased beyond 1.25 to 1.30, a decrease in fruit quality occurred.

There was no apparent relationship between the phosphorus content of the foliage and storage quality of the fruit.

Results with the Spy Variety

The results of foliage analysis, fruit quality and spoilage index are recorded in Table 6. There is a very highly significant correlation ($r = -.9439^{**}$) between the nitrogen level in the foliage and the quality score of the fruit. There does not appear to be a sharp break in this relationship, although the marked decrease in quality occurs with nitrogen levels above 2.0 to 2.1 per cent, as shown in Figure 8. Although there was no direct relationship apparent between the potassium level in the foliage and fruit quality there was a significant negative correlation ($r = -.5703^{**}$) between the nitrogen-potassium ratio and quality as shown in Figure 9. There is a considerable decrease in quality with a nitrogen-potassium ratio greater than 1.2.

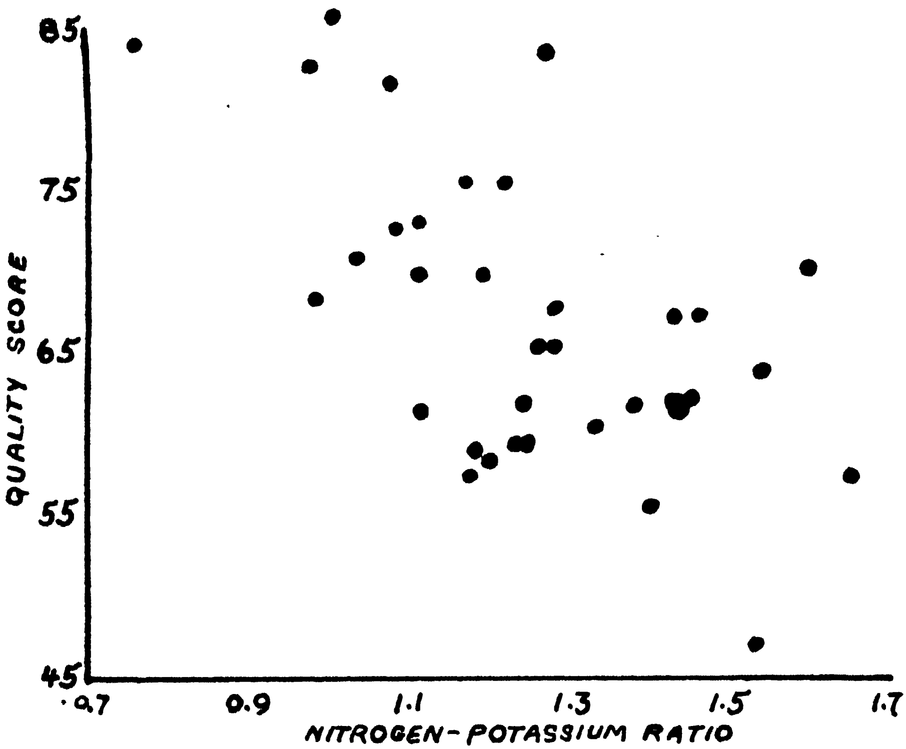

FIGURE 9. Relation between nitrogen-potassium ratio and Spy storage quality in a uniform orchard block—1949.

SUMMARY AND CONCLUSIONS

The relation between foliage analyses for nitrogen, potassium, phosphorus, magnesium and keeping quality of McIntosh and Spy apples was studied with material derived from two main sources: from individual trees located in a considerable number of different orchards under a variety of management and fertilizer use and secondly, from trees in a uniform orchard block receiving specific fertilizer treatments.

In the first phase of the study with the McIntosh variety a significant negative correlation was obtained between the nitrogen content of the foliage and the quality score of the fruit. When only nitrogen was considered the quality of the fruit was reduced if the nitrogen level in the foliage exceeded 2.0 to 2.1 per cent. There was a positive trend between potassium levels in the foliage up to 1.7 per cent of dry matter and fruit quality. Fruit from trees whose foliage had a nitrogen-potassium ratio of not more than 1.25 had a significantly higher quality score than samples where the nitrogen-potassium ratio was above this figure.

No relationship was established between the phosphorus content of the foliage and quality score or spoilage index of the fruit.

In the Spy variety no definite relationships were established between foliage analyses and fruit quality.

In the second phase of the study the application of increasing increments of nitrogen brought about a steady increase of nitrogen in the foliage, accompanied by a considerable decrease of potassium and phosphorus. The widening of the N/K ratio was associated with a decrease in fruit quality. Progressive increments of superphosphate had no consistent effect upon the phosphorus, nitrogen or potassium content of the foliage.

In both the McIntosh and Spy varieties there was a definite negative correlation between the level of nitrogen in the foliage and the quality score of the fruit. As in the first phase of the study the critical level appeared to be approximately 2.0 to 2.1 per cent. There was also a decrease in the fruit quality if the nitrogen-potassium ratio exceeded 1.25.

There was no apparent relationship between the phosphorus content of the foliage and the storage quality of the fruit.

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